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# **Biocompatibility Testing of Medical Devices – Standards Specific Information for the Accreditation Scheme for Conformity Assessment (ASCA) Program**

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## **Draft Guidance for Industry, Accreditation Bodies, Testing Laboratories, and Food and Drug Administration Staff**

***DRAFT GUIDANCE***

**This draft guidance document is being distributed for comment purposes only.**

**Document issued on September 23, 2024.**

You should submit comments and suggestions regarding this draft document within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <https://www.regulations.gov>. Submit written comments to the Dockets Management Staff, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD 20852. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions about this document, contact the ASCA Program at [ASCA@fda.hhs.gov](mailto:ASCA@fda.hhs.gov). For questions about this document regarding CBER-regulated devices, contact the Office of Communication, Outreach, and Development (OCOD) at 1-800-835-4709 or 240-402-8010, or by email at [ocod@fda.hhs.gov](mailto:ocod@fda.hhs.gov).

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**When final, this guidance will supersede Biocompatibility Testing of Medical Devices – Standards Specific Information for the Accreditation Scheme for Conformity Assessment (ASCA) Pilot Program, issued September 25, 2020.**



U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Devices and Radiological Health  
Center for Biologics Evaluation and Research

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See additional PRA statement in [Section VII](#) of this guidance

# Preface

## Public Comment

You may submit electronic comments and suggestions at any time for Agency consideration to <https://www.regulations.gov>. Submit written comments to the Dockets Management Staff, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD 20852. Identify all comments with the docket number FDA-2019-D-3805. Comments may not be acted upon by the Agency until the document is next revised or updated.

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1 **Biocompatibility Testing of Medical**  
2 **Devices – Standards Specific**  
3 **Information for the Accreditation**  
4 **Scheme for Conformity Assessment**  
5 **(ASCA) Program**

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7 **Draft Guidance for Industry,**  
8 **Accreditation Bodies, Testing**  
9 **Laboratories, and**  
10 **Food and Drug Administration Staff**

11  
12 *This draft guidance, when finalized, will represent the current thinking of the Food and*  
13 *Drug Administration (FDA or Agency) on this topic. It does not establish any rights for*  
14 *any person and is not binding on FDA or the public. You can use an alternative approach*  
15 *if it satisfies the requirements of the applicable statutes and regulations. To discuss an*  
16 *alternative approach, contact the FDA staff or Office responsible for this guidance as*  
17 *listed on the title page.*

18 **I. Introduction**

19 This guidance provides information on how the Biological Evaluation of Medical Devices  
20 standards are incorporated into the Accreditation Scheme for Conformity Assessment  
21 Program (hereafter referred to as the ASCA Program). The ASCA Program is described in  
22 FDA's draft guidance [The Accreditation Scheme for Conformity Assessment \(ASCA\)](#)  
23 [Program](#).

24  
25 For the current edition of the FDA-recognized consensus standards included in the ASCA  
26 Program, see the [FDA Recognized Consensus Standards Database](#). For more information  
27 regarding use of FDA-recognized consensus standard in regulatory submissions, please refer  
28 to the FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket](#)  
29 [Submissions for Medical Devices](#) and [Standards Development and the Use of Standards in](#)  
30 [regulatory Submissions Reviewed in the Center for Biologics Evaluation and Research](#).

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32 In general, FDA’s guidance documents, including this guidance, do not establish legally  
33 enforceable responsibilities. Instead, guidances describe the Agency’s current thinking on a  
34 topic and should be viewed only as recommendations, unless specific regulatory or statutory  
35 requirements are cited. The use of the word *should* in Agency guidance means that  
36 something is suggested or recommended, but not required.

37 **II. Scope**

38 This guidance includes the following:

39  
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- a list of the FDA-recognized consensus standards and test methods included in the ASCA Program for biocompatibility testing of medical devices,
- assessment and accreditation of testing laboratories by ASCA-recognized accreditation bodies,
- program specifications for the FDA-recognized consensus standards and test methods in the ASCA Program for biocompatibility testing of medical devices, and
- recommended premarket submission contents specific to FDA-recognized consensus standards and test methods for biocompatibility testing of medical devices when testing is conducted by an ASCA-accredited testing laboratory.

50 FDA’s guidance [The Accreditation Scheme for Conformity Assessment \(ASCA\) Program](#)  
51 describes how accreditation bodies, testing laboratories, device manufacturers, and FDA staff  
52 participate in the ASCA Program as well as how FDA-recognized consensus standards and  
53 test methods are selected and how program specifications are developed.

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Please see FDA’s guidance [Use of International Standard ISO 10993-1, “Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process”](#) for recommendations on biocompatibility testing to support a premarket submission. The ASCA Program for biocompatibility testing of medical devices does not include certain types of devices that require customized<sup>1</sup> sample preparation and/or testing methodologies, absorbable devices, *in situ* polymerizing devices, liquid devices, creams, gels, hydrogel devices, and devices containing nanomaterials. All biocompatibility testing under the ASCA Program should be conducted according to 21 CFR 58 Good Laboratory Practices (GLP) for Nonclinical Laboratory Studies regulations. If biocompatibility testing is not conducted in compliance with 21 CFR 58 GLP regulations, it is considered outside of the ASCA Program.<sup>2</sup>

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<sup>1</sup> Sample preparation and/or test methodologies conducted per sponsor’s specific request (i.e., on a case-by-case basis) that deviate from the testing laboratory’s procedures (e.g., sample preparation Standard Operating Procedures (SOPs), test method SOPs) are considered customization and therefore are excluded from ASCA.

<sup>2</sup> If such biocompatibility testing data are submitted in regulatory submissions, additional information may be needed. For more information, please see FDA’s guidance entitled [Use of International Standard ISO 10993-1, “Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process”](#).



67 **III. List of FDA-Recognized Consensus Standards and**  
68 **Test Methods in the ASCA Program for Biocompatibility**  
69 **Testing of Medical Devices**

70 Biological evaluation assesses the biocompatibility-related risks of medical devices with  
71 direct and/or indirect contact with human tissue. When biocompatibility testing is needed as  
72 part of a premarket submission to FDA to address biocompatibility-related risks, the selected,  
73 cross-cutting biological evaluation standards listed below<sup>3</sup> are relevant to many device  
74 manufacturers and the device types are of significant public health importance.

- 75
- 76 • ASTM F756: *Standard Practice for Assessment of Hemolytic Properties of Materials*
  - 77 • ASTM F720: *Standard Practice for Testing Guinea Pigs for Contact Allergens:*  
78 *Guinea Pig Maximization Test*
  - 79 • ISO 10993-4: *Biological evaluation of medical devices – Part 4: Selection of tests for*  
80 *interactions with blood*
  - 81 • ISO 10993-5: *Biological evaluation of medical devices – Part 5: Tests for in vitro*  
82 *cytotoxicity*
  - 83 • ISO 10993-10: *Biological evaluation of medical devices – Part 10: Tests for skin*  
84 *sensitization*
  - 85 • ISO 10993-11: *Biological evaluation of medical devices – Part 11: Tests for systemic*  
86 *toxicity*
  - 87 • USP <151>: *Pyrogen Test*
  - 88 • ISO 10993-12: *Biological evaluation of medical devices – Part 12: Sample*  
89 *preparation and reference materials*
  - 90 • ISO 10993-23<sup>4</sup>: *Biological evaluation of medical devices – Part 23: Tests for*  
91 *irritation*
  - 92 • ISO 10993-2: *Biological evaluation of medical devices – Part 2: Animal welfare*  
93 *requirements*

---

<sup>3</sup> The currently recognized versions of the standards included in the ASCA Program are listed in the [FDA Recognized Consensus Standards database](#). However, some test methods from these standards may not be included in the ASCA Program.

<sup>4</sup> Irritation tests are moved from ISO 10993-10 fourth edition and published as ISO 10993-23.

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94 It is important to note that the eligible test methods included in the ASCA Program for  
95 biocompatibility testing of medical devices are:

FDA-Recognized Consensus Standard	Test method(s)
ISO 10993-4*	SC5b-9 Complement Activation <sup>5</sup> using a U.S. marketed ELISA kit
ISO 10993-4 and ASTM F756	Direct and Indirect Hemolysis
ISO 10993-5	MEM Elution Cytotoxicity
ISO 10993-10 <sup>6</sup>	Closed Patch Sensitization
ISO 10993-23 <sup>7</sup>	Dermal Irritation, Intracutaneous Reactivity Irritation
ISO 10993-10 and ASTM F720 <sup>8</sup>	Guinea Pig Maximization Sensitization
ISO 10993-11	Acute Systemic Toxicity
ISO 10993-11 and USP 151	Material-Mediated Pyrogenicity
ISO 10993-2	Animal welfare requirements for animal tests
ISO 10993-12	Sample preparation for all test types

96 \* See also ISO/TS 10993-20 for information on when complement activation should be considered for  
97 anaphylaxis (Table 2, Hypersensitivity Column).

98  
99 The extent of FDA recognition (complete or partial) is provided in the Supplemental  
100 Information Sheet (SIS) for each standard listed in the [FDA Recognized Consensus](#)  
101 [Standards Database](#). The SIS provides additional information to consider when using FDA-  
102 recognized consensus standards, such as relevant guidance documents that provide clarity on  
103 FDA recommendations for testing to support premarket submissions.

## 104 **IV. Assessment and Accreditation of Testing Laboratories**

### 105 **A. Scope of Assessments**

106 Clause 7 of ISO/IEC 17011: *Conformity assessment – Requirements for accreditation bodies*  
107 *accrediting conformity assessment bodies* (hereafter referred to as “ISO/IEC 17011”)  
108 describes processes by which accreditation bodies assess testing laboratories. To maintain  
109 conformance to ISO/IEC 17011, an accreditation body assesses a sample of the scope of  
110 accreditation of its accredited testing laboratories at least every two years.<sup>9</sup> An accreditation  
111 body also performs a reassessment of its accredited testing laboratories before the end of the  
112 accreditation cycle that confirms the competence of each testing laboratory for all the

<sup>5</sup> See FDA’s guidance Use of International Standard ISO 10993-1, “Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process” which recommends SC5b-9 complement activation using an established ELISA method for in vitro complement activation testing.

<sup>6</sup> We support the principles of the “3Rs,” to reduce, refine, and replace animal use in testing when feasible. We encourage sponsors to consult with us if they wish to use a non-animal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method. See generally: <https://www.fda.gov/science-research/advancing-regulatory-science/vi-modernizing-safety-testing>

<sup>7</sup> *Ibid*

<sup>8</sup> *Ibid*

<sup>9</sup> See 7.9.3 of ISO/IEC 17011: 2017: *Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies*.

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113 requirements of the FDA-recognized consensus standards and test methods within the  
114 laboratory's scope of accreditation.<sup>10</sup>

115

116 When assessing a testing laboratory under the ASCA Program, an ASCA-recognized  
117 accreditation body is expected to assess all (and not a sample of) biological evaluation  
118 standards and test methods to ensure competence across the testing laboratory's scope of  
119 *ASCA Accreditation*.

120

121 When assessing testing laboratories under the ASCA Program, accreditation bodies should  
122 assess the conformance to all FDA-recognized consensus standards clauses and ASCA  
123 Program specifications (see [Section IV.E.](#) of this guidance) that are applicable to the test  
124 methods included in the testing laboratory's scope of *ASCA Accreditation*.

125

126 This assessment should include:

127

- 128 i) assessing the adequacy of the testing laboratory's established procedures (including  
129 Standard Operating Procedures (SOPs), protocol templates, forms, worksheets and  
130 training) that are adequate<sup>11</sup> to address all applicable FDA-recognized consensus  
131 standards clauses and ASCA specifications in the testing laboratory's scope for  
132 ASCA (and to confirm that any controlled documents that reference both ASCA and  
133 non-ASCA methods specify which are/are not ASCA),
- 134 ii) observing the testing laboratory's personnel while they conduct sample preparation  
135 and test methods (e.g., certain phases of each test included in the scope of ASCA  
136 accreditation) and assessing the competency of technical personnel (including use of  
137 SOPs, work instructions, forms, worksheets) during these observations,
- 138 iii) assessing training documents to ensure adequate training is provided to meet ASCA  
139 specifications (e.g., proficiency check, mock study, classroom training, on-the-job  
140 training), and ensuring the completion of all training for technical personnel prior to  
141 initiation of ASCA testing, and
- 142 iv) verifying proper authorization and dates of all changes, deviations, and amendments  
143 to controlled documents.

144

145 Similarly, ASCA-recognized accreditation bodies are expected to plan and perform a  
146 reassessment of all FDA-recognized consensus standards and test methods, including the  
147 ASCA Program specifications detailed below, within the testing laboratory's scope of  
148 *ASCA Accreditation* to confirm the competence of a testing laboratory prior to the end of  
149 the assessment cycle.

150

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<sup>10</sup> See 7.9.4 of ISO/IEC 17011: 2017: *Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies*.

<sup>11</sup> The term adequate means that procedures are clear, complete, and can be followed by a trained individual. Section IV.D., 7.2 c) of this guidance describes specifications that a test procedure needs to include or specify.

151 **B. Competency of Accreditation Body’s Technical**  
152 **Assessors**

153 The ASCA-recognized accreditation body should ensure the competency of all technical  
154 assessors for the ASCA Program. Additionally, the ASCA-recognized accreditation body  
155 should maintain records demonstrating the following:

- 156
- 157 i. All technical assessors are knowledgeable in all of the FDA-recognized  
158 consensus standards and test methods under the ASCA Program, the ASCA  
159 Program specifications in [Section IV.E.](#) of this guidance, as well as FDA’s  
160 guidance [Use of International Standard ISO 10993-1, “Biological evaluation of](#)  
161 [medical devices – Part 1: Evaluation and testing within a risk management](#)  
162 [process,”](#) and
  - 163 ii. All technical assessors have<sup>12</sup>a Bachelor’s or higher degree in a scientific  
164 discipline and one of the following, at a minimum:
    - 165 • two years relevant experience in medical device biocompatibility testing,
    - 166 • two years relevant experience with in vivo testing, or
    - 167 • two years relevant experience with in vitro testing (e.g., cell biology  
168 testing).

169

170 The accreditation body will also ensure that technical assessors attend all relevant FDA  
171 ASCA-related trainings prior to providing any accreditation to testing laboratories under the  
172 ASCA Program, including:

- 173
- 174 • notifying FDA of new personnel who need FDA training,
  - 175 • not assigning personnel in ASCA-related activities until training is complete,  
176 and
  - 177 • committing to maintaining competence and capacity for the requested scope  
178 of *ASCA Recognition*.

179 **C. Accreditation by ASCA-Recognized Accreditation**  
180 **Bodies**

181 ASCA-recognized accreditation bodies should only accredit testing laboratories that have  
182 demonstrated competence in biocompatibility testing and provided adequate evidence of  
183 conformance to all applicable FDA-recognized consensus standards clauses and test methods  
184 and ASCA Program specifications as described in [Section IV.E.](#) of this guidance for the  
185 requested scope of *ASCA Accreditation*. Any nonconformance or deficiencies should be  
186 adequately addressed by the testing laboratory before the ASCA-recognized accreditation  
187 body grants the accreditation. The accreditation body will maintain records and supporting  
188 evidence from the testing laboratory to demonstrate how a nonconformance or deficiency  
189 identified during the assessment has been adequately addressed by the testing laboratory.

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<sup>12</sup> Alternative approaches may be considered if the accreditation body provides documentation and detailed rationales to FDA for why the alternative approach demonstrates equivalency to the specifications listed here.

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190 ASCA-recognized accreditation bodies should not accredit a testing laboratory under the  
191 ASCA Program if the testing laboratory has any of the following issues:

- 192
- 193 i) conducts fraudulent activities or has issues with test data integrity,
  - 194 ii) is non-compliant with or in violation of 21 CFR 58,
  - 195 iii) copies or paraphrases ASCA specifications or FDA-recognized consensus  
196 standards clauses into the controlled documents without detailed procedures on  
197 implementation of the ASCA Program specifications and FDA-recognized  
198 consensus standards clauses,
  - 199 iv) lacks document control,
  - 200 v) lacks evidence demonstrating conformance to all applicable FDA-recognized  
201 consensus standards clauses and ASCA specifications in the requested scope of  
202 accreditation for ASCA, or
  - 203 vi) has animal welfare<sup>13</sup> issues (e.g., unresolved animal welfare violations based on a  
204 national authority’s inspection).

205 **D. Scope of ASCA Accreditation Issued by Accreditation**  
206 **Bodies**

207 Once testing laboratories have been assessed by an ASCA-recognized accreditation body to  
208 ISO/IEC 17025 and the ASCA Program specifications identified in [Section IV.E.](#) of this  
209 guidance document, the accreditation body will issue a proposed scope of *ASCA*  
210 *Accreditation* to the testing laboratory (see sample in Appendix D in the [ASCA Program](#)  
211 [guidance document](#)). The testing laboratory should work with their accreditation body to  
212 ensure that the FDA-recognized consensus standards and test methods are accurately and  
213 clearly listed in the ASCA section of the scope of *ASCA Accreditation*.

214

215 For biocompatibility testing, not all test methods included in FDA-recognized consensus  
216 standards are included in the ASCA Program. Accreditation bodies and testing laboratories  
217 should consult [Section III](#) of this guidance and the [FDA-Recognized Consensus Standards](#)  
218 [Database](#) for a full listing of FDA-recognized consensus standards and test methods included  
219 in the ASCA Program (note that individual test methods are contained within standards). The  
220 document number of the Standard Operating Procedure (SOP) that the testing laboratories  
221 follow for each test method should also be listed under “Test Method & Procedure” in the  
222 proposed scope of *ASCA Accreditation*.

223

---

<sup>13</sup> Animal Welfare Act, 7 U.S.C. § 2131 et seq., as amended. 2013; Animal Welfare Regulations, 9 CFR Chapter 1, Subchapter A, Parts 1, 2, and 3. 2004; Health Research Extension Act of 1985, Public Law 99-158 November 20, 1985; Office of Laboratory Animal Welfare, National Institutes of Health. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Bethesda, MD; 2015; National Research Council. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press; 2011

224 **E. ASCA Program Specifications for Biocompatibility**  
225 **Testing of Medical Devices**

226 The ASCA Program specifications in this section provide expectations for the accreditation  
227 of testing laboratories for the biocompatibility testing of medical devices under the ASCA  
228 Program. ASCA-recognized accreditation bodies, following the processes of ISO/IEC  
229 17011, accredit testing laboratories to all relevant elements of ISO/IEC 17025: 2017:  
230 *General requirements for the competence of testing and calibration laboratories* (hereafter  
231 referred to as “ISO/IEC 17025”) as well as the ASCA Program specifications identified in  
232 this section. Throughout the ASCA Program specifications below, the term will is used to  
233 convey that testing laboratories undergoing assessments by ASCA recognized accrediting  
234 bodies are able to provide supportive documentation or information demonstrating  
235 competence to each of the ASCA Program specifications below.

236  
237 ASCA-recognized accreditation bodies will assess the testing laboratories to the  
238 specifications below. In addition, all testing should be conducted considering the  
239 recommendations in FDA’s guidance [Use of International Standard ISO 10993-1,](#)  
240 [“Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk](#)  
241 [management process.”](#)  
242

243 For readability and ease of reference, the numbering and nomenclature (including the term  
244 requirements)<sup>14</sup> below correspond to the numbering and nomenclature of clauses/subclauses  
245 in ISO/IEC 17025.

246  
247 **ISO/IEC 17025 Clause 4 “General requirements”**

248 **4.1 Impartiality**

249 If any services, such as consulting, design, or research, are offered by the testing laboratory,  
250 it will have a policy and procedure for maintaining impartiality through separation of those  
251 services from its testing activities.

252 A device manufacturer’s internal testing laboratory will have policies and procedures that  
253 specifically ensure and protect the impartiality of the laboratory to test or otherwise evaluate  
254 devices manufactured by the laboratory’s parent organization and, if applicable, other device  
255 manufacturers without regard to the impact of the test results on the parent organization’s  
256 business interests.

257 **4.2 Confidentiality**  
258

259 There are no additional specifications above those set forth in ISO/IEC 17025.

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<sup>14</sup> Some definitions within voluntary FDA-recognized consensus standards refer to “requirements.” FDA’s references to them for the ASCA Program do not make them legal or regulatory requirements unless specifically identified as such.



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260 **ISO/IEC 17025 Clause 5 “Structural requirements”**

261

262 There are no additional specifications above those set forth in ISO/IEC 17025.

263 **ISO/IEC 17025 Clause 6 “Resource requirements”**

264 6.1 General

265 There are no additional specifications above those set forth in ISO/IEC 17025.

266 6.2 Personnel

- 267 a) The testing laboratory will maintain competent technical personnel that are  
268 knowledgeable in the appropriate test method(s) for the requested scope of  
269 accreditation and have relevant experience and education that meet the criteria as  
270 described in [Appendix A](#) of this guidance.
- 271 b) The testing laboratory’s management will be knowledgeable in applicable aspects of  
272 the FD&C Act and 21 CFR regulations pertinent to the oversight of medical devices  
273 and the criteria set out in ISO/IEC 17025 and ASCA Program specifications. The  
274 testing laboratory further will maintain a list of laboratory managers and contact  
275 information.
- 276 c) The testing laboratory will:
- 277 1) Document and maintain a training program for new and previously trained  
278 technical personnel, which will include the proper procedures for applying  
279 new/updated test procedures and performing required tests;
- 280 2) Provide new and previously trained technical personnel relevant test-specific  
281 requalification training (e.g., cytotoxicity subjective scoring) every 6-12 months,  
282 or when test standards or procedures are updated or developed, as well as when  
283 responsibilities have changed;
- 284 3) Conduct training on a periodic basis through application of training approaches,  
285 such as on-the-job training and formal classroom training, as appropriate;
- 286 4) Document and maintain records of training demonstrating that technical personnel  
287 who participate in the conduct of ASCA testing have been trained and evaluated  
288 to be competent in the performance of each ASCA test. The training includes the  
289 ability to follow test-related standard operating procedures (SOPs) and  
290 documentation, and in person hands-on training. Training may also include  
291 classroom (or online) training. Testing laboratories further agree to have  
292 predefined criteria to qualify that technical personnel (technicians and study  
293 directors) can perform assigned tasks related to the tests under the scope of *ASCA*  
294 *Accreditation*, and for when retraining will be needed (e.g., when technicians  
295 have not conducted the test or assigned task for a specified period of time, change  
296 in assigned activities, when procedures are updated, as a corrective or preventive  
297 action in response to non-conformance to procedures), and follow-up actions  
298 needed in the case of mock study or proficiency check failures;

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- 299 5) Establish procedures for periodic internal test lab proficiency checks<sup>15</sup> of  
300 technicians (e.g., blind scoring of negative and positive controls for MEM elution  
301 assays) for the tests performed under the ASCA Program with subjective  
302 analyses; and
- 303 6) Maintain records demonstrating trainers have qualifications and at least 2 years’  
304 experience (routinely performing each relevant ASCA test) to train the technical  
305 personnel (i.e., technicians or trainees) who will perform the ASCA tests.
- 306 d) The testing laboratory will have procedures to establish how test and control samples  
307 are prepared and training (e.g., training manuals, training records) that includes the  
308 following:
- 309 1) Procedures for device preparation, including:
- 310 i. Cutting samples (if appropriate) including examples of devices that should  
311 not be cut and how to handle previously unexposed surfaces when cutting  
312 samples as well as documentation (e.g., photographs) of any particle  
313 generation prior to extraction;
- 314 ii. Determination of device surface area for extraction ratio including how  
315 surface area is calculated (e.g., calculation based on dimensional  
316 measurement using formula, calculation based on engineering drawings,  
317 sponsor provided surface area based on engineering drawings), and the  
318 volume required to complete the study;
- 319 iii. Use of non-standard surface area approaches (e.g., porous devices),
- 320 iv. Exclusion of non-contacting components from extraction;
- 321 v. Selection of representative portions for direct contact hemocompatibility  
322 studies (i.e., hemolysis, complement activation);
- 323 vi. Selection of extraction time and temperature. The following is a list of  
324 acceptable extraction times and temperatures:
- 325 •  $(37\pm 1)^{\circ}\text{C}$  for  $(24\pm 2)\text{h}^{\text{a}}$   
326 •  $(37\pm 1)^{\circ}\text{C}$  for  $(72\pm 2)\text{h}^{\text{b}}$   
327 •  $(50\pm 2)^{\circ}\text{C}$  for  $(72\pm 2)\text{h}^{\text{c}}$   
328 •  $(70\pm 2)^{\circ}\text{C}$  for  $(24\pm 2)\text{h}^{\text{c}}$   
329 •  $(121\pm 2)^{\circ}\text{C}$  for  $(1\pm 0.1)\text{h}^{\text{c}}$   
330  
331 h = hour
- 332 Note a: for MEM elution cytotoxicity testing of devices with limited (less  
333 than or equal to 24 h) contact.

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<sup>15</sup> For hemolysis, complement activation, and material-mediated pyrogenicity testing, there are no ASCA specifications for a periodic proficiency check.



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- 334 Note b: for MEM elution cytotoxicity testing of devices with prolonged  
335 (>24 h to 30 days (d)) or long-term (>30 d) contact.
- 336 Note c: for testing (other than MEM elution cytotoxicity) of devices if  
337 devices do not contain heat labile or heat sensitive materials (e.g., drugs)  
338 or if devices do not contain materials that may have the potential to  
339 undergo deformation or material configuration/structural change at such  
340 temperature.
- 341 vii. Selection of extraction vehicle. Common extraction vehicles for each test  
342 method within the ASCA Program are listed in the “Extraction Vehicle”  
343 section of Appendices C~K of this guidance; and
- 344 viii. Solvent compatibility pre-test between the selected solvent and the test  
345 article as well as when solvent compatibility pre-test should be conducted  
346 (e.g., when softening or deformation of the device material occurs in the  
347 presence of the solvent).
- 348 2) Assessment and documentation of changes (e.g., photographs) after extraction to  
349 sample (e.g., color changes, integrity, swelling) or extract conditions (e.g., pH,  
350 particles/precipitates, color changes, or turbidity);
- 351 3) General and/or test-specific follow-up procedures when changes are noted (e.g.,  
352 extract settling techniques to allow particle-free IV injections);
- 353 4) Use of non-standard extraction approaches (e.g., fluid path approaches,  
354 approaches for extremely large devices, procedures to maintain contact with  
355 extraction vehicle);
- 356 5) Handling of extracts prior to testing (e.g., filtration, centrifugation, storage time  
357 and temperature); and
- 358 6) Extract storage (e.g., used immediately, refrigerated at 2 to 8 °C for no longer than  
359 24 hours without visible precipitation).
- 360 e) In addition, for each specific test method, the testing laboratory agrees to establish a  
361 training program (e.g., training manual, training records) to demonstrate how training  
362 will be conducted and how technician competency will be evaluated (e.g., for in vivo  
363 tests, number of animals used in training technicians and acceptance criteria for task  
364 completion). At a minimum, the training program will address the test method-  
365 specific ASCA specifications in each associated Appendix (e.g., Appendices C-K).
- 366 f) For in vivo studies, as part of the general animal care and observation training, the  
367 testing laboratory agrees to establish a training program (e.g., training manual,  
368 training records) to demonstrate how training will be conducted and how technician  
369 competency will be evaluated (e.g., acceptance criteria). This training program and  
370 the procedures related to animal care and handling will address the following, at a  
371 minimum:
- 372 1) Test-specific animal model selection criteria (e.g., species/strain, age, weight, sex,  
373 and source);

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- 374 2) Animal identification and traceability;  
375 3) Species- and test-specific animal handling and restraining techniques;  
376 4) Test-specific acclimatization and quarantine;  
377 5) Animal housing and husbandry;  
378 6) Environmental conditions (e.g., lighting cycles, temperature, and relative  
379 humidity);  
380 7) Body weight measurement;  
381 8) Species-specific in life observations (e.g., cage accidents, behavior changes,  
382 decline in health, seizures, weight loss, breathing difficulties), criteria for  
383 assessment, data capture, test-specific frequency of observations, and when  
384 veterinarian examination should be requested;  
385 9) Test-specific data documentation, calculations, analysis and result interpretation  
386 (including test-specific assessment of borderline scores, and re-challenge or re-  
387 test criteria, when applicable); and  
388 10) Criteria for technician retraining.

389 6.3 Facilities and environmental conditions

390 Lab personnel should be aware of the FD&C Act and regulations as applicable to medical  
391 device manufacturers. Under 21 CFR 820.50, Purchasing Controls, medical device  
392 manufacturers must communicate as part of contracted work any environmental conditions  
393 necessary for the proper conduct of testing done under the scope of accreditation. In  
394 addition, testing laboratories should have policies and procedures in place to implement 21  
395 CFR part 58, Good Laboratory Practices (GLP), for Nonclinical Laboratory Studies.

396 Testing laboratories conducting biocompatibility testing under the ASCA Program need to be  
397 previously inspected for 21 CFR 58 (GLP) by FDA. FDA will consider alternatives if  
398 sufficient evidence (e.g., meeting OECD Mutual Acceptance of Data (MAD) criteria  
399 available at <https://www.oecd.org/chemicalsafety/testing/mutualacceptanceofdatamad.htm>) is  
400 provided to demonstrate testing laboratory's compliance with GLP requirements.

401 Testing laboratories agree to establish procedures for animal care per 21 CFR 58 and ISO  
402 10993-2 for in vivo tests in their scope for ASCA.

403 6.4 Equipment

- 404 a) The testing laboratory will ensure that all equipment used for testing and evaluating  
405 devices is available and in proper working order for the requested scope of  
406 accreditation.  
407 b) The testing laboratory will ensure that its procedures address adding, deleting,  
408 modifying, or maintaining information in equipment records in an accurate and timely  
409 manner, and specify the personnel responsible for these tasks.

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- 410 c) The testing laboratory will ensure that its procedures specify the steps for establishing  
411 calibration intervals for each type or item of equipment, and specify criteria, steps,  
412 and approvals for extending the calibration interval of an instrument.
- 413 d) The testing laboratory will have procedures to examine the effects of equipment  
414 operation outside the equipment tolerances or study specified limits (e.g., temperature  
415 excursions) on test results. The procedures identify the personnel responsible for such  
416 examination of the equipment (e.g., technicians) and determination of acceptability  
417 with respect to test validity (e.g., study directors/toxicologists), specify their  
418 responsibilities, and provide the steps for determining if the equipment variation  
419 would impact the study results, including:
- 420 1) Determining whether the effects are unacceptable (including the accept/reject  
421 criteria),
  - 422 2) Identifying the conducted tests affected,
  - 423 3) Analyzing the results impacted for these particular tests, and
  - 424 4) Determining whether retesting is required.

425 6.5 Metrological traceability

- 426 a) Testing laboratories agree to use specified methods and/or standards that clearly  
427 describe the following:
- 428 1) Calibration to three decimal places for spectrophotometer absorbance readings for  
429 hemolysis and complement activation, and
  - 430 2) Particle ranges for calibration of coulter counter use for cell counting.
- 432 b) If test-specified positive, negative, and/or reference controls are no longer able to  
433 distinguish between positive and negative responses, the testing laboratory will have  
434 procedures to qualify new lots or new suppliers of the controls. The procedure should  
435 address how each test-specific control (positive/negative/reagent, if applicable) will  
436 be qualified for each test method. For example, material specifications for purchasing  
437 controls could be established based on Certificate of Analyses (CoAs) so that only  
438 controls that meet the pre-established specifications (e.g., purity, reagent grade,  
439 appearance) can be used for testing. If verification testing is used to qualify the  
440 controls, testing laboratories need to establish procedures and document test methods  
441 used to qualify the controls. In addition, the frequency of qualification testing,  
442 acceptance criteria, and expiration date for the controls should be specified in the  
443 procedure. [Appendix L](#) in this guidance includes a table for the controls and reagents  
444 that could impact the validity of a test, and for which purchase control and/or  
445 verification testing specifications need to be established.
- 446 c) The testing laboratory agrees that controls (positive/negative/reagent, if applicable)  
447 will meet assay-specific acceptance criteria. For example, testing laboratory will  
448 establish the procedure to monitor the performance of positive and negative control

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449 materials (e.g., trending analysis for in vitro testing, test control investigation if pre-  
450 specified criteria are not met).

451 d) The testing laboratory agrees that, when concurrent positive controls are not  
452 conducted with the test article (i.e., sensitization testing), biannual testing (i.e., within  
453 3 months of the test article) will be conducted to confirm the ability of the test system  
454 to detect a positive sensitization response. If it is determined that the periodic positive  
455 control is no longer valid, all testing conducted after the last validated positive control  
456 run cannot be submitted as part of the ASCA Program.

457 6.6 Externally provided products and services

458 a) The testing laboratory will ensure that any subcontractors utilized to conduct testing  
459 under the scope of *ASCA Accreditation* are ASCA-accredited testing laboratories for  
460 the selected tests.

461 **ISO/IEC 17025 Clause 7 (“Process requirements”)**

462 7.1 Review of requests, tenders and contracts

463 There are no additional specifications to those set forth in ISO/IEC 17025.

464 7.2 Selection, verification and validation of methods

465 a) The testing laboratory agrees that its management system will include procedures  
466 governing the development, maintenance, and use of test procedures (including  
467 associated documents such as test data forms and checklists). These management  
468 system procedures include steps for:

469 1) Identifying the personnel responsible for developing, reviewing, and maintaining  
470 these documents,

471 2) Specifying the frequency of review by technical personnel and management

472 3) Ensuring consistency with applicable standard(s),

473 4) Ensuring test modifications are reviewed by personnel who are competent to the  
474 applicable standard(s), and

475 5) Identifying and documenting the types of modifications to the test procedures that  
476 do not need to be reviewed by FDA for confirmation prior to implementation, if  
477 included in the test lab application.

478 b) The testing laboratory further agrees that changes (either at the request of study  
479 sponsor or initiated by the test lab) to any ASCA procedures will be confirmed with  
480 FDA and its Accreditation Body prior to implementation. For example:

481 i. Changes to sample for retesting to achieve a “passing” result

482 ii. pH adjustments,

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- 483           iii.    Sample filtration or other extract manipulation,  
484           iv.    Removal or modification of documentation associated with color, turbidity  
485                or particles in the test extract, or swelling/degradation of the test article  
486           v.    Frequency of non-concurrent control testing,  
487           vi.    Changes to acceptance criteria outside the validated/qualified laboratory-  
488                specific limits (e.g., for complement activation where the standard  
489                methods do not specify acceptable limits),  
490           vii.   Changes to data calculations and presentation, if applicable (e.g.,  
491                hemolytic index, irritation index, complement activation plots),  
492           viii.   Changes in the criteria for re-challenge or retesting,  
493           ix.    Changes in the criteria for reportable adverse clinical observations or  
494                animal deaths, and  
495           x.    Any unanticipated changes.<sup>16</sup>
- 496    c)    The testing laboratory agrees that test procedures will include or specify, as  
497           appropriate, the following:
- 498           1)    Unique identification, including title, document number, revision, and effective  
499                date,  
500           2)    Specific test equipment to use along with their required ratings,  
501           3)    Warnings/caution statements to alert the operators of potential hazards,  
502           4)    Normal and any unusual ambient conditions (including tolerances) for tests,  
503           5)    Test data to be obtained and recorded,  
504           6)    Objective acceptance criteria for results including the essential performance  
505                required to be maintained,  
506           7)    Testing techniques (i.e., test methods) required to ensure consistent results,  
507           8)    Instructions on test conduct, including equipment operation, reagent preparation,  
508                cell line and animal handling, techniques, preparation of test samples (including  
509                instructions for sample traceability during testing, if applicable), conduct of each  
510                step of the test, data recording, and scoring assessment procedures,  
511           9)    Deviations from the SOP, as well as any equipment deviations and discussion of  
512                why deviations will not impact the validity of the study results, and  
513           10)   If the procedure is written for both ASCA testing and non-ASCA testing, then the  
514                portion of the procedure excluded from ASCA testing should be clearly specified.

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<sup>16</sup> Unanticipated changes to the ASCA-test related controlled documents (e.g., test method SOPs, protocol templates, test report templates, work instructions, general SOPs that address ASCA Program specifications, data collection worksheets, training information) are the changes not defined in the documents submitted for ASCA accreditation application.

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515 11) Detailed instructions to address test method-specific ASCA specifications in each  
516 associated Appendix (e.g., Appendices C-K).

517 d) The testing laboratory will ensure that relevant contextual information from the  
518 intended use of the device is collected in the controlled documents (e.g., sample  
519 submission form) and considered in the testing to ensure that the types of biological  
520 evaluation assessments recommended by FDA are considered based on tissue type  
521 and duration of contact with the device. In addition, relevant information from the  
522 device manufacturers' essential performance specifications, including any thermal  
523 properties of the device materials and the relevant clinical use conditions, are also  
524 collected and considered to ensure that the test procedures (e.g., extraction  
525 temperature, time, and extraction vehicles) are compatible with the device, and

526 e) The testing laboratory will ensure that each test procedure adequately addresses all  
527 the applicable specifications of the standard for the devices being tested.

528 7.3 Sampling

529 a) The testing laboratory agrees that the procedure(s) for sample preparation will meet  
530 the specifications of ISO 10993-12 and FDA's guidance [Use of International](#)  
531 [Standard ISO 10993-1, "Biological evaluation of medical devices--Part 1: Evaluation](#)  
532 [and testing within a risk management process"](#).<sup>17</sup> The testing laboratory will also  
533 establish a training program (e.g., training manuals, training records). The procedures  
534 and the training should address the following::

535 1) Use of surface area/extraction volume ratio (unless mass/extraction volume ratio  
536 results in equivalent or higher amount of test sample),

537 2) Use of mass/extraction volume ratio for powders,

538 3) How sample extraction ratio (e.g., 6 cm<sup>2</sup>/ml, 3 cm<sup>2</sup>/ml) will be selected,

539 4) How extraction ratio of 1.25 cm<sup>2</sup>/ml will be selected for elastomer devices (i.e.,  
540 thickness greater than 1mm). If extraction ratio of 1.25 cm<sup>2</sup>/ml is used for  
541 elastomeric devices less than 0.5 mm thick or devices between 0.5 and 1 mm  
542 thick, include procedures to specify when it is acceptable. For example, if  
543 extractions conducted at higher surface area to extraction volume ratios (i.e., 6  
544 cm<sup>2</sup>/ml, 3 cm<sup>2</sup>/ml) result in the entire volume being absorbed or insufficient  
545 volume remains for testing,

546 5) No dilutions of extract or test solutions, unless required for dose-dependent  
547 cytotoxicity studies,

548 6) No filtration/centrifugation,

549 7) No pH/osmolality adjustment,

550 8) No complete device dissolution during extraction,

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<sup>17</sup> Available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-international-standard-iso-10993-1-biological-evaluation-medical-devices-part-1-evaluation-and>

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- 551 9) Documentation of any color changes, turbidity or particles in the extract,  
552 10) How representative portions are proportionally selected for testing, if the test  
553 system cannot accommodate all of the direct and indirect tissue contacting device  
554 components, to include documentation of what was excluded,  
555 11) How extraction vehicle volume will be determined and documented for absorbent  
556 devices (e.g., spongy or porous devices) including details on absorbency capacity  
557 determination,  
558 12) How sample extraction ratios will be selected for devices having multiple  
559 components with different thicknesses,  
560 13) How components with different types and durations of contact will be separated  
561 for sample preparation and testing,  
562 14) Situations when pooled component samples (with same or different types or  
563 duration of tissue contact) will be allowed,  
564 15) Inclusion of only tissue contacting components (unless procedure describes how  
565 inclusion of non-tissue contacting components will be addressed in determination  
566 of extraction ratios),  
567 16) Submersion of large devices completely in extraction vehicle,  
568 17) How extractions will be conducted for devices containing fluid path components  
569 (e.g., complete fill, partial fill with agitation (ISO 10993-12 surface/volume ratio),  
570 partial fill with agitation (other surface/volume ratio)), and  
571 18) That the following types of devices are excluded for the ASCA Program: devices  
572 that require customized<sup>18</sup> sample preparation and/or testing methodologies,  
573 absorbable devices, in situ polymerizing devices, liquid devices, creams, gels,  
574 hydrogel devices, and devices containing nanomaterials.

575 7.4 Handling of test or calibration items

576 There are no additional specifications to those set forth in ISO/IEC 17025.

577 7.5 Technical records

578 There are no additional specifications to those set forth in ISO/IEC 17025.

579 7.6 Evaluation of measurement uncertainty

580 There are no additional specifications to those set forth in ISO/IEC 17025.

581 7.7 Ensuring the validity of results

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<sup>18</sup> Sample preparation and/or test methodologies conducted per sponsor's specific request (i.e., on a case-by-case basis) that deviate from the testing laboratory's procedures (e.g., sample preparation Standard Operating Procedures (SOPs), test method SOPs) are considered customization and therefore are excluded from ASCA.

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- 582 a) To confirm the validity of the testing methods, any test-specified positive, negative,  
583 and/or reference controls allow for distinguishing between positive and negative  
584 responses. The testing laboratory agrees that pre-defined criteria for  
585 positive/negative/reference control values will meet the test method-specific ASCA  
586 specifications in each associated Appendix in this document.
- 587 b) Testing laboratories agree to establish procedures for management of unexpected results  
588 and non-conformance quality events (e.g., non-conformance to test method SOPs,  
589 protocols, and work instructions) encountered during testing and operational activities.  
590 General procedures should be established for root cause investigation and analysis to  
591 support corrective and preventive actions.

592 7.8 Reporting of results

- 593 a) The testing laboratory agrees that it will have procedures to record all required  
594 information in ISO/IEC 17025 for each test conducted, including the following:
- 595 1) Test procedure(s) and test standard(s) used,
  - 596 2) Product or component(s) tested,
  - 597 3) Test equipment used for testing, measurement, or review (including the  
598 equipment's ratings and accuracies, unless otherwise readily available),
  - 599 4) Date of the test(s). For example, periodic controls may have different test dates,
  - 600 5) Test report number, including revision number and amendment date, if applicable,  
601 and any related sub-contracted test report number(s),
  - 602 6) Names of the personnel performing the test(s) and the names of all supervisory  
603 personnel involved in the study and for biological studies, the signature of the  
604 study director and quality assurance unit personnel (i.e., per 21 CFR part 58,  
605 Good Laboratory Practices for Nonclinical Laboratory Studies, requirements),
  - 606 7) The test conditions as specified by the test standard, if applicable, (e.g., required  
607 voltage, power, temperature, or humidity for the test),
  - 608 8) Sample preparation:
    - 609 i. images of device (or representative portion, if full device is not used) prior  
610 to and post sample preparation,
    - 611 ii. documentation of device components that are sampled, and those that are  
612 not sampled, and
    - 613 iii. use of subdivision/cutting.
  - 614 9) Extraction conditions, if applicable:
    - 615 i. extraction vehicle, time, temperature, means of agitation, and test  
616 article/vehicle ratio,
    - 617 ii. storage time and temperature prior to application to the test system, and



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- 618                   iii.    images of vehicle post-extraction (color, cloudiness, presence of  
619                   particulates).
- 620           10) Sample manipulation:
- 621                   i.    filtration, centrifugation, dilution, pH adjustment, osmolality adjustment  
622                   or other deviations from the sampling procedures,
- 623           11) Any deviations from the laboratory’s ASCA accepted procedures as well as any  
624           amendments to the test report,
- 625           12) Test results to include:
- 626                   i.    opinions and interpretations included in a test report,  
627                   ii.   all of the applicable data required by the laboratory's procedures, and  
628                   iii.   a statement that testing was conducted according to 21 CFR 58 Good  
629                   Laboratory Practices for Nonclinical Laboratory Studies regulations.
- 630           b) The testing laboratory agrees that testing conducted by subcontractors will also  
631           comply with the above test report specifications, as applicable, and
- 632           c) The testing laboratory agrees that the complete test report and an ASCA Summary  
633           Test Report will be submitted to the client at the end of testing activities.

634   7.9 Complaints

635   There are no additional specifications than those set forth in ISO/IEC 17025.

636   7.10 Nonconforming work

637   There are no additional specifications than those set forth in ISO/IEC 17025.

638   7.11 Control of data and information management

639   There are no additional specifications than those set forth in ISO/IEC 17025.

640   **ISO/IEC 17025 Clause 8 (“Management system requirements”)**

641   8.1 Options

642   Regardless of the option selected (i.e., Option A or Option B), the testing laboratory will  
643   maintain SOPs and any relevant ASCA test-related documents (e.g., test method SOPs,  
644   protocol templates, test report templates, work instructions, general SOPs that address ASCA  
645   Program specifications, data collection worksheets, training information) applicable to any  
646   biological evaluation of medical device standards or test methods.

647

648 **V. Recommendations for Testing Laboratory**  
649 **Participation in the ASCA Program**

650 **A. Demonstration of Competency**

651 Testing laboratories that participate in the ASCA Program are expected to establish  
652 procedures and provide evidence to demonstrate conformance to all standards clauses and  
653 ASCA Program specifications ([Section IV.E.](#) of this guidance) that are applicable to the test  
654 methods included in the testing laboratory’s scope of *ASCA Accreditation*. Testing  
655 laboratories should develop detailed procedures to describe how the FDA-recognized  
656 consensus standards clauses, test methods and ASCA Program specifications are  
657 implemented in their laboratories.

658  
659 To seek *ASCA Accreditation*, a testing laboratory should provide all documents listed in  
660 Appendix B of the draft [ASCA Program guidance document](#) and any additional application  
661 elements related to biocompatibility testing of medical device standards and test methods in  
662 the requested scope of *ASCA Accreditation*. All documents need to be in English and need to  
663 be clear, complete, and detailed enough such that a trained individual could follow them.

664

665 **B. Test Plan Development**

666 FDA encourages testing laboratories that participate in the ASCA Program to work with  
667 device manufacturers to develop a test plan on what specific biocompatibility assessments  
668 will be conducted based on the type and duration of device contact that is consistent with  
669 recommendations as described in Attachment A of FDA’s guidance [Use of International](#)  
670 [Standard ISO 10993-1, “Biological evaluation of medical devices - Part 1: Evaluation and](#)  
671 [testing within a risk management process.”](#) Testing laboratories are expected to include a  
672 general procedure (e.g., a flowchart) in the controlled documents to specify how type and  
673 duration of device contact will determine recommended biocompatibility assessment.

674

675 To prepare the ASCA Declaration of Conformity (ASCA DOC) for a premarket submission,  
676 the device manufacturer may consult with the testing laboratory for information relevant to  
677 the “Limitations on Validity of DOC” section (See [Appendix B](#) for the Example ASCA DOC  
678 for Biological Evaluation of Medical Devices Standards in the ASCA Program).

679 **VI. Premarket Submission Contents for FDA-Recognized**  
680 **Consensus Standards and Test Methods in the ASCA**  
681 **Program for Biocompatibility Testing of Medical Devices**

682 FDA recommends that the following be included in any premarket submission that contains  
683 biocompatibility testing conducted by an ASCA-accredited testing laboratory.

684 **A. Cover Letter**

685 FDA’s recommendations regarding the content to be included in a cover letter for a  
686 premarket submission containing testing results from an ASCA-accredited testing laboratory  
687 are provided in FDA’s guidance [The Accreditation Scheme for Conformity Assessment](#)  
688 [\(ASCA\) Program](#).

689 **B. ASCA Declaration of Conformity (ASCA DOC)**

690 Section IV.A. of FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in](#)  
691 [Premarket Submissions for Medical Devices](#) recommends contents for a declaration of  
692 conformity (DOC) to an FDA-recognized consensus standard. For biocompatibility testing  
693 conducted under the ASCA Program from an ASCA-accredited testing laboratory, FDA  
694 recommends the device manufacturer include the following additional items in an ASCA  
695 DOC:

- 696
- Date(s) the testing was conducted.
  - 697
  - Location(s) where the testing was conducted.
  - 698
  - Confirmation that the FDA-recognized consensus standards (and specific test  
699 methods) used during testing were within the laboratory’s scope of *ASCA*  
700 *Accreditation* and not subject to any temporary labeling constraints as a result of a  
701 suspension of *ASCA Accreditation* at the time testing was conducted. If the relevant  
702 standard (and specific test method) was impacted by a suspension of *ASCA*  
703 *Accreditation*, the ASCA DOC should include an explanation of how this suspension  
704 may or may not affect the testing results.
  - 705
  - Limitations on the validity of the ASCA DOC:
    - 706
    - How the test article compares with the device provided in this premarket  
707 submission (including selection of “representative” devices/portions).
    - 708
    - Details about how any concerns communicated by the test lab were resolved,
    - 709
    - How any observations and/or degradations during testing were resolved,
    - 710
    - Whether any adverse or unusual findings as described in the list in [Section VI.C.](#)  
711 of this guidance occurred and, if so, rationale for acceptability, and
    - 712
    - If there is additional data or documentation that support any of the limitations on  
713 the validity of the ASCA DOC, a reference identifying where to find this  
714 information should be provided in the ASCA DOC.

715 An example ASCA DOC is provided in *Appendix B* of this guidance.

716 **C. Supplemental Documentation**

717 An ASCA Summary Test Report should be submitted for all testing conducted under the  
718 ASCA Program. Example ASCA Summary Test Reports are provided in Appendices C-K of  
719 this guidance. Note that the ASCA-accredited testing laboratory provides the ASCA  
720 Summary Test Report to the device manufacturer who then includes it with its own ASCA

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721 DOC in a premarket submission to FDA. Depending on the information provided in the  
722 ASCA DOC or the ASCA Summary Test Report, FDA may or may not need to review the  
723 complete test report for biocompatibility testing,<sup>19</sup> and the testing laboratory and/or device  
724 manufacturer may also be requested to provide a rationale to support a decision on a  
725 premarket submission.

726

727 Under the ASCA Program, FDA generally will accept results from ASCA-accredited testing  
728 laboratories when the FDA-recognized consensus standard and test methods are within the  
729 testing laboratory’s scope of *ASCA Accreditation* at the time of testing. Circumstances where  
730 FDA might request and review additional information related to testing from an ASCA-  
731 accredited testing laboratory are described in the bulleted points of Section XIII.A. of FDA’s  
732 guidance [The Accreditation Scheme for Conformity Assessment \(ASCA\) Program](#).

733

734 The ASCA Program processes and policies enhance confidence in testing results only when  
735 specific test methods and acceptance criteria are used. For example, FDA reviews a copy of  
736 SOPs and any relevant ASCA test-related documents (e.g., test method SOPs, protocol  
737 templates, test report templates, work instructions, general SOPs that address ASCA Program  
738 specifications, data collection worksheets, training information) for testing laboratories that  
739 apply for a scope of *ASCA Accreditation* that includes biocompatibility testing. This review  
740 provides FDA an understanding of how testing is conducted, thereby providing confidence in  
741 the competence of ASCA-accredited testing laboratories. Depending on the specific device or  
742 intended use, deviations or amendments relative to the testing documentation submitted to  
743 FDA during the *ASCA Accreditation* application process<sup>20</sup> may be appropriate. In such cases,  
744 FDA recommends a complete test report be included in the premarket submission. FDA also  
745 recommends a complete test report be included in the premarket submission for specific  
746 circumstances when (based on FDA’s review experience) results may indicate a potential  
747 concern. These cases are noted below and, in each example, ASCA Summary Test Report  
748 (*refer to the test method-specific Appendices of this guidance*).

749

- 750 • If test article was prepared per the ASCA Test Article Prep SOP specified on the  
751 ASCA Summary Test Report (*refer to the test method-specific Appendices of this*  
752 *guidance*) with deviations/amendments (e.g., filtering, extract manipulation, pH  
753 adjustment).
- 754 • If extraction solvent, ratio, or conditions other than those specially called out in the  
755 example ASCA Summary Test Report (*refer to the test method-specific Appendices*  
756 *of this guidance*) were used.
- 757 • If there were any changes in color/turbidity or particles in the test article and/or  
758 extract OR there was swelling/degradation of the test article.

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<sup>19</sup> A complete test report for biocompatibility testing is described in Attachment E of FDA’s guidance [Use of International Standard ISO 10993-1, “Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process”](#)

<sup>20</sup> Testing that includes deviations (and for which a ASCA DOC would not be appropriate) does not meet the criteria for inclusion in the ASCA Program as described in Section XII.B. of the guidance titled [The Accreditation Scheme for Conformity Assessment \(ASCA\) Program](#).

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- 802
- If testing was conducted per the ASCA Test Method SOP specified on the ASCA Summary Test Report (*refer to the test method-specific Appendices of this guidance*) and per 21 CFR 58 with deviations/amendments.
  - If the test article was not the entire final finished device or a representative sample selection per the ASCA Test Article Prep SOP.
  - If extraction is conducted under static conditions or intermittent agitation.
  - For irritation- intracutaneous reactivity (ISO 10993-23) testing:
    - if the overall score differences between the test and control are greater than one (i.e., per ISO 10993-23:2021, Clause 7.3.7), or if there were non-zero results for any of the sodium chloride control sites in any animal or results greater than 1 for any of the oil control sites in any animal at any timepoint, and
    - if adverse clinical findings or animal deaths occurred.
  - For cytotoxicity – MEM Elution (ISO 10993-5) testing: if there were non-zero results for the test article, vehicle control or negative control, or if there were results less than 3 for the positive control at any timepoint.
  - For dermal irritation (ISO 10993-23):
    - if the types of test article are other than powder, solid sample, or test article extracts,
    - if test and control article application methods are other than those specifically called out in the example ASCA summary test report (*refer to Appendix E of this guidance*),
    - if other exposure periods (e.g., repeated exposure, single exposure greater than 24 hours) are used,
    - if the primary irritation score is calculated using different timepoints besides 24 hours, 48 hours, and 72 hours, if there were any non-zero test or control (e.g., direct contact control: gauze; extract test control: sodium chloride or oil) results at any time point, and
    - if adverse clinical findings or animal deaths occurred.
  - For guinea pig maximization sensitization (ISO 10993-10 and ASTM F720):
    - if the Magnusson and Kligman grades of 1 or greater are observed in the test or the sodium chloride and oil vehicle control groups (i.e., per ISO 10993-10:2021, Clause 6.5.6),
    - if differences in source, strain, treatment methods, or timing of the positive control occurred, and
    - if adverse clinical findings or animal deaths occurred.
  - For closed patch sensitization (ISO 10993-10):
    - if the types of test article are other than powder, solid sample, or test article extracts,
    - if test and control article application methods are other than those specifically called out in the example ASCA Summary Test Report (*refer to Appendix G of this guidance*),
    - if the Magnusson and Kligman grades of 1 or greater observed in the test group, provided grades of less than 1 are seen in negative control animals (i.e.,

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- 803 per ISO 10993-10:2021, Clause 6.6.6), or the sodium chloride and oil vehicle  
804 controls are > Grade 0,
- 805 • if differences in source, strain, treatment methods, or timing of the positive  
806 control occurred, and
  - 807 • if adverse clinical findings or animal deaths occurred.
  - 808 • For acute systemic toxicity (ISO 10993-11):
    - 809 • if any test or control animals died or had any adverse clinical findings, and
    - 810 • if any test animals had a body weight loss greater than 10%.
  - 811 • For material-mediated pyrogenicity (ISO 10993-11 and USP 151):
    - 812 • if any rabbit has a baseline temperature exceeding 39.8°C, or if any rabbit has  
813 a temperature rise  $\geq 0.5^\circ\text{C}$ , and
    - 814 • if adverse clinical findings or animal deaths occurred.
  - 815 • For direct and indirect hemolysis (ISO 10993-4 and ASTM F756):
    - 816 • if direct contact was used and a diluent other than Magnesium and Calcium  
817 Free PBS was used (i.e., per ASTM F756-17, Section 3.1.10),
    - 818 • if direct contact was used and an exposure ratio other than those specially  
819 called out in the example ASCA Summary Test Report (*refer to Appendix J of*  
820 *this guidance*) was used (i.e., per ASTM F756-17, Section 9.2.1),
    - 821 • if the test article red blood cell pellet after centrifugation was visually  
822 different in color or size (e.g., larger or very small) compared to the pellet for  
823 the negative control,
    - 824 • if one of the following occurs: (1) the negative and positive controls did not  
825 perform as expected, (2) the negative control, test article, and blank had  
826 absorbance values of 0.000 for all replicates, (3) any replicate of the negative  
827 control, test article, or blank samples had a “Blank Corrected % Hemolysis”  
828 value less than -1% (e.g., -1.5%), or (4) the final Hemolytic Index of the test  
829 article  $\geq 2\%$ , and
    - 830 • if the total hemoglobin concentration of the diluted blood is outside of the  
831 range of 9-11 mg/ml.
  - 832 • For SC5b-9 complement activation (ISO 10993-4):
    - 833 • if a test medium other than those specifically called out in the example ASCA  
834 Summary Test Report (*refer to Appendix K of this guidance*) was used,
    - 835 • if exposure ratio or conditions other than those specially called out in the  
836 example ASCA Summary Test Report (*refer to Appendix K of this guidance*)  
837 were used, and
    - 838 • if test medium, negative, positive, and comparator controls did not perform as  
839 expected, or there was a statistically significant increase in SC5b-9 for test  
840 article compared to negative or comparator controls.

841 **VII. Paperwork Reduction Act of 1995**

842

843 This guidance contains information collection provisions that are subject to review by the  
844 Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (44  
845 U.S.C. 3501-3521).

846

847 The time required to complete this information collection is estimated<sup>21</sup> to average 95 hours  
848 per response for accreditation bodies and 47 hours for testing laboratories. Send comments  
849 regarding this burden estimate or suggestions for reducing this burden to:

850

851 FDA PRA Staff,  
852 Office of Operations,  
853 Food and Drug Administration,  
854 [PRStaff@fda.hhs.gov](mailto:PRStaff@fda.hhs.gov)

855

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. The OMB control number for this information collection is 0910-0889 (To find the current expiration date, search for this OMB control number available at <https://www.reginfo.gov>).

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<sup>21</sup> Rounded to the nearest whole number.

859 **Appendix A: Relevant Experience and Educational**  
 860 **Requirements<sup>22</sup> for Technical Personnel that Conduct**  
 861 **ASCA Tests**

<b>Personnel Type</b>	<b>Experience</b>	<b>Education</b>
Technicians performing in vivo tests	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• 1 year of relevant test experience with each standard test included in the ASCA Program to which technicians are assigned</li> <li>• demonstrated proficiency through completion minimally of 25 tests to which technicians are assigned demonstrated proficiency through completion minimally of 25 phases to which technicians are assigned and as outlined in each study specific training.</li> </ul>	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• Bachelor’s or associate degree in relevant science areas to the biocompatibility testing included in the ASCA Program</li> <li>• a high school degree, and at least one of the following laboratory technician accreditations<sup>23</sup>:                             <ul style="list-style-type: none"> <li>○ Assistant Laboratory Animal Technician (ALAT),</li> <li>○ Laboratory Animal Technician (LAT)</li> <li>○ Laboratory Animal Technologist (LATG).</li> </ul> </li> </ul>
Technicians performing in vitro tests	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• 1 year of relevant test experience with each standard test included in the ASCA Program to which technicians are assigned</li> <li>• demonstrated proficiency through completion minimally of 25 tests to which technicians are assigned</li> <li>• demonstrated proficiency through completion minimally of 25 phases to which technicians are assigned and as outlined in each study specific training</li> </ul>	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• Bachelor’s or associate degree in relevant science areas to the biocompatibility testing included in the ASCA Program</li> <li>• A high school degree and previous laboratory experience (at a minimum, a total of 3 years of experience AND 75 tests) on the specific phase or the entire in vitro test included in the ASCA Program</li> </ul>

<sup>22</sup>Alternative approaches may be considered if, as part of the ASCA accreditation application, the testing laboratory provides documentation and detailed rationales to FDA for why the alternative approach demonstrates equivalency to the specifications listed here.

<sup>23</sup> Other animal technician certifications/diplomas that are equivalent to ALAT, LAT, and LATG (e.g., similar “certification test” specifications that cover, at a minimum, animal husbandry, health and welfare, and facility administration and management) may substitute the requirements for ALAT, LAT and LATG certifications for technicians, if the testing laboratory’s established procedures specify the documentation used to demonstrate equivalency (e.g., scope of a “certification test,” “certification test” specifications, handbook materials for the “certification test,” sample of the “certification test”).



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<p>Technicians performing any test specific (e.g., for complement activation) sample preparation</p>	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• 1 year of sample preparation experience with the relevant standard test included in the ASCA Program to which technicians are assigned</li> <li>• demonstrated proficiency through completion of sample preparation for minimally 25 tests to which technicians are assigned</li> </ul>	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• Bachelor’s or associate degree in science</li> <li>• A high school degree and previous laboratory experience (at a minimum, a total of 3 years of experience AND 75 tests) on sample preparation with the relevant standard test included in the ASCA Program to which technicians are assigned</li> </ul>
<p>Technicians performing sample preparation that is applicable for various tests (e.g., technicians in general sample preparation lab who prepare samples/extracts for various tests)</p>	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• 1 year sample preparation experience with any standard test included in the ASCA Program</li> <li>• demonstrated proficiency through completion of sample preparation for minimally 25 of any of the standard tests in the ASCA Program</li> </ul>	<p>Meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• Bachelor’s or associate degree in science</li> <li>• A high school degree and previous laboratory experience (at a minimum, a total of 3 years of experience AND 75 tests) on sample preparation with any standard test included in the ASCA Program.</li> </ul>
<p>Study directors</p>	<p>2 years of relevant test experience with each standard test and meet one of the following, at a minimum:</p> <ul style="list-style-type: none"> <li>• direction of at least 25 studies in each relevant test</li> <li>• management of 25 studies with someone who has directed at least 25 studies in each relevant test</li> </ul>	<p>Bachelor’s or higher degree in scientific discipline</p>

863 **Appendix B: Example ASCA DOC for Biological**  
864 **Evaluation of Medical Devices Standards in the ASCA**  
865 **Program**

866 *Note: This example is intended to illustrate elements expected in an ASCA DOC. The content*  
867 *of an ASCA DOC expands on the content of a DOC described in FDA’s guidance*  
868 [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical](#)  
869 [Devices](#). *Submitters should include an ASCA DOC as part of its premarket submission, when*  
870 *including ASCA testing (i.e., an ASCA Summary Test Report).*

871  
872 **Responsible Party**

873 Name of entity responsible for DOC: \_\_\_\_\_

874 Address of entity responsible for DOC: \_\_\_\_\_

875

876 **Product/Device Identification**

*All identifying information for the product/device (e.g., product code(s), device marketing name(s), model number(s), etc.).*

877

878 **Statement of Conformity**

879 The test results demonstrate that the device is in conformity with the standard(s) listed  
880 below:<sup>24</sup>

881

882 1. Title of Standard(s)<sup>25</sup>: (e.g., *ISO 10993-10 Third edition 2021-11 Biological*  
883 *evaluation of medical devices – Part 10: Tests for skin sensitization*) \_\_\_\_\_

884

• FDA Recognition #(s): (e.g., 2-296)

885

• Options Selected

886

Standard(s) included no options

887

Standard(s) included options

888

List of options selected in standards:

889

MEM Elution Cytotoxicity: ISO 10993-5:2009 Clause 8.2 and Table 1

890

SC5b-9 Complement Activation: ISO 10993-4:2017 Annex B using US  
891 marketed ELISA kit

892

Direct Hemolysis: ISO 10993-4:2017 Annex D and ASTM F756

893

Indirect Hemolysis: ISO 10993-4: 2017 Annex D and ASTM F756

894

Guinea Pig Maximization Sensitization (GPMT): ISO 10993-10:2021  
895 Clause 6.5 and ASTM F720-17

<sup>24</sup> See section 514(c)(3)(A)(i) of the FD&C Act, cited in Section IV.A.(3)(f) of FDA’s guidance.

<sup>25</sup> A device manufacturer may declare conformity to multiple standards and test methods evaluated by a single testing laboratory within the ASCA Program on the ASCA DOC.

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- 896  Closed Patch Sensitization: ISO 10993-10:2021 Clause 6.6  
897  Dermal Irritation: ISO 10993-23:2021 Clause 7.2  
898  Intracutaneous Reactivity Irritation: ISO 10993-23:2021 Clause 7.3  
899  Acute Systemic Toxicity: ISO 10993-11:2017 Clause 5  
900  Material-Mediated Pyrogenicity: ISO 10993-11:2017 Annex G and USP  
901 <151>  
902 • Testing Laboratory Name: (e.g., Testing Laboratory ABC)  
903 • ASCA Testing Laboratory Identification Number (as applicable): (e.g., ASCA001)  
904 • Testing Location(s): (e.g., 1234 Example Road, Silver Spring, MD 20993)  
905 • Testing Date(s): (e.g., Sep 1, 2024 – Sep 15, 2024)  
906 • ASCA Accreditation Status on the Date(s) of Testing:  
907  Standard(s) (and particular test methods) were not in testing laboratory’s scope  
908 of ASCA Accreditation  
909  Standard(s) (and particular test methods) were in testing laboratory’s scope of  
910 ASCA Accreditation  
911  ASCA Accreditation was not suspended  
912  ASCA Accreditation was suspended

*If ASCA Accreditation was suspended during the testing date(s), a description of reasons for suspension and their impact on testing results should be provided. FDA may need to review additional information and/or ask questions to determine whether the test results can be used to support a decision on a premarket submission.*

**Note:** *if the testing laboratory’s ASCA Accreditation was suspended or withdrawn at the time of testing, an ASCA DOC may not be submitted for the suspended or withdrawn FDA-recognized consensus standards and test methods. However, the submitter may submit a DOC as outlined in FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#).*

- 913 • Supplemental Documentation (see [Section VI.C.](#) of this guidance for specific  
914 recommendations):  
915  Supplementary documentation is not included  
916  Supplementary documentation is included at the following location within the  
917 submission, and I have checked that there are no differences regarding  
918 protocol and data between the complete test report and the supplemental  
919 documentation: (e.g., MEM Elution Cytotoxicity (ISO 10993-5) ASCA  
920 Summary Test Report located in Appendix A of this premarket  
921 submission)  
922  
923  
924  
925

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926 **Limitations on Validity of DOC**

*Description of any limitation on the validity of the ASCA DOC (e.g., how long the declaration is valid, what was tested, or concessions made about the testing outcomes) including a reference to relevant locations in the premarket submission. For testing from an ASCA-accredited test laboratory, this should include, at a minimum:*

- *Information on how the test article for each test compares with the device provided in this premarket submission<sup>26</sup> (including, selection of “representative” devices/portions) can be found at the following location in this premarket submission: (e.g., Section V, pages 45-50)*
- *Information on how any concerns communicated by the test lab were resolved can be found at the following location in this premarket submission: (e.g., Appendix D of this premarket submission)*
- *Information on how any observations and/or degradations during testing were resolved can be found at the following location in this premarket submission: (e.g., Appendix D of this premarket submission)*
- *A statement that the device/test article does not require customized sample preparation and/or testing methodologies, and is not an absorbable or in situ polymerizing device, liquid device, cream, gel, hydrogel device, or a device containing nanomaterials, as these types of materials are not eligible for biocompatibility testing under the ASCA Program*

927

928 **Signature**

929 Printed name: \_\_\_\_\_

930 Function within entity responsible for DOC: \_\_\_\_\_

931

932

933 Signature \_\_\_\_\_ Date \_\_\_\_\_

934

<sup>26</sup> Please see FDA’s guidance [Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process"](#) for considerations regarding the use of medical devices in their final finished form or a representative test article for biocompatibility testing.

935 **Appendix C: Test Method-Specific ASCA Specifications**  
936 **and Summary Test Report: Irritation – Intracutaneous**  
937 **Reactivity (ISO 10993-23)**

938 **A. ASCA Specifications: Intracutaneous Reactivity**  
939 **Irritation (ISO 10993-23)**

940 **ISO/IEC 17025 Subclause 6.2(e)**

941 The procedures, documentation and training program will address the following, at a  
942 minimum:

- 943 i. Shaving techniques (e.g., to avoid razor burn),  
944 ii. Application of test samples,  
945 iii. Injection technique and signs to confirm appropriate injection location,  
946 iv. Injection site scheme and marking,  
947 v. Differentiation for source of redness (e.g., true irritation versus possible  
948 irritation from shaving),  
949 vi. Clinical observations (e.g., cage side observation, skin site observation,  
950 and presence of adverse events), criteria for assessment, data capture, and  
951 frequency (e.g., minimum daily),  
952 vii. Evaluation criteria and basis for retest,  
953 viii. Data documentation, calculations, analysis and result interpretation,  
954 ix. Minimally, biannual periodic technician proficiency check of positive  
955 response scoring (in live animals at least once annually). The proficiency  
956 check procedure should specify, at a minimum, the following:  
957  
958 - positive and negative controls used,  
959 - number of positive and negative controls, number of animals and/or  
960 number of images used (if images are used for proficiency check),  
961 - protocol used to conduct the study, and  
962 - pass/fail criteria. For example, comparison of technician and trainer  
963 scores and criteria for acceptable level of agreement: for each  
964 individual site, and the overall score (e.g., irritant vs. non-irritant).  
965  
966 x. Criteria for technician retraining, if needed.  
967

968 **ISO/IEC 17025 Subclause 7.7(a)**

969 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
970 values will be as follows:

- 971 i. each of five sodium chloride control sites in each animal at all timepoints is  
972 Grade 0,  
973 ii. each of five oil control sites in each animal at all timepoints is  $\leq$  Grade 1,  
974 iii. confirmation of assay sensitivity by running positive control study at least  
975 every six months or tracking that at least one test article positive result has  
976 occurred within the previous six months.

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977 **B. Example ASCA Summary Test Report: Intracutaneous**  
978 **Reactivity Irritation (ISO 10993-23)**

979 *Note: This example is intended to illustrate the supplemental documentation that would*  
980 *accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing*  
981 *laboratory to the device manufacturer.*

982

983 **Administrative Information**

984 1. Testing Laboratory Name:

985 2. ASCA Testing Laboratory Identification Number:

986 3. Testing Location(s):

987 4. Testing Date(s):

988 5. ASCA Accreditation Status on the Date(s) of Testing:

989  Standard (and particular test method) was \*NOT\* in testing laboratory's scope of  
990 ASCA Accreditation<sup>27</sup>

991  Standard (and particular test method) was in testing laboratory's scope of ASCA  
992 Accreditation

993  ASCA Accreditation was not suspended

994  ASCA Accreditation was suspended

*Description of reasons for suspension and their impact on testing results.*

995

996 **ASCA Test Article Prep SOP#:** [ASCATAPrep(date/version)]

997  Test Article was prepared per the above protocol (no deviations/amendments), or

998  Test Article was prepared per the above protocol, with the following  
999 deviations/amendments<sup>28</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

1000 **Test Article:**

1001  Entire final finished device

1002  Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]

1003

1004  Other:<sup>29</sup> [DESCRIBE]

<sup>27</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of ASCA Accreditation.

<sup>28</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>29</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

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1005 **Extraction Solvent:**

- 1006  0.9% Sodium Chloride (SC)  
1007  Cotton Seed Oil (CSO)/Sesame Oil (SO)  
1008  Other:<sup>30</sup> [DESCRIBE]  
1009 \_\_\_\_\_

1010 **Extraction Ratio:**

- 1011  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*  
1012  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*  
1013  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*  
1014  0.2 g/ml (for powder devices)  
1015  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
1016 test article as compared to surface/volume ratio) [*Provide information on comparison*  
1017 *when mass/volume ratio versus surface area/volume ratio is used.*]  
1018 \*Note: For absorbent device only: [Specify surface area of test article and the total  
1019 volume of extraction vehicle used taking into account the additional volume from  
1020 absorbency determination.]  
1021  Other:<sup>31</sup> [DESCRIBE]  
1022 \_\_\_\_\_

1022 **Extraction Conditions:**

- 1023  37°C, 72 h  
1024  50°C, 72 h  
1025  70°C, 24 h  
1026  121°C, 1 h  
1027  Other:<sup>32</sup> [DESCRIBE]  
1028 \_\_\_\_\_

1029 **Agitation During Extraction:**

- 1030  Extraction with continuous agitation or circulation  
1031  Extraction under static conditions or intermittent agitation<sup>33</sup>: [DESCRIBE and PROVIDE  
1032 JUSTIFICATION]  
1033 \_\_\_\_\_

1034 **Fluid Path Extractions:**

- 1035  For fluid path devices or components (where fluids contact the channels in the device or  
1036 component, and then the fluid enters the body), the extraction was conducted using protocols  
1037 specific to fluid path, with the following approach:<sup>34</sup>  
1038  Complete fill with agitation  
1039  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

---

<sup>30</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>31</sup> *Ibid*

<sup>32</sup> *Ibid*

<sup>33</sup> *Ibid*

<sup>34</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report is requested in addition to the ASCA Summary Test Report.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

- 1040  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]  
1041  Other: [SUMMARIZE APPROACH]

1042

1043 **Extract Observations:**

- 1044  The test article and extract DID NOT change color, and the extract DID NOT appear  
1045 turbid or have particles.  
1046  There were changes in color/turbidity or particles in the test article and/or extract OR  
1047 there was swelling/degradation of the test article.<sup>35</sup>

1048

1049 **ASCA Test Method SOP #:** [ASCAIntracut(date/version)]

- 1050  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
1051 or  
1052  Test was conducted per the above protocol and 21 CFR 58, with the following  
1053 deviations/amendments.<sup>36</sup>

*Description of deviations/amendments*

1054 **Results:**<sup>37</sup>

<sup>35</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>36</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>37</sup> The complete test report should be included with ASCA Summary Test Report, if the overall score differences between the test and control are greater than one (i.e., per ISO 10993-23:22021, Clause 7.3.7), or if there were non-zero results for any of the sodium chloride control sites in any animal, or results greater than 1 for any of the oil control sites in any animal at any timepoint.



**Contains Nonbinding Recommendations**

**Draft – Not for Implementation**

	<b>Test Article</b>	<b>24 h Results</b>	<b>48 h Results</b>	<b>72 h Results</b>	<b>Conclusions</b>
Animal 1	SC Test	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	Performed as expected
	SC Control	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	Performed as expected
Animal 2	SC Test	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	Performed as expected
	SC Control	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	Performed as expected
Animal 3	SC Test	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	Performed as expected
	SC Control	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	ER: 0/0/0/0/0 ED: 0/0/0/0/0	Performed as expected
Animal 1	SO Test	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/0/1/1/1 ED: 0/0/0/0/0	ER: 1/0/1/1/1 ED: 0/0/0/0/0	Performed as expected
	SO Control	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/1/0/0/1 ED: 0/0/0/0/0	Performed as expected
Animal 2	SO Test	ER: 1/1/1/1/1 ED: 0/0/1/0/0	ER: 1/1/1/1/0 ED: 0/0/1/0/0	ER: 1/1/1/1/0 ED: 0/0/0/0/0	Performed as expected
	SO Control	ER: 1/1/1/1/0 ED: 0/0/1/0/0	ER: 1/1/1/0/1 ED: 0/0/0/0/0	ER: 1/1/0/0/0 ED: 0/0/0/0/0	Performed as expected
Animal 3	SO Test	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/1/1/1/1 ED: 0/0/0/0/0	Performed as expected
	SO Control	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/1/1/1/1 ED: 0/0/0/0/0	ER: 1/1/1/1/1 ED: 0/0/0/0/0	Performed as expected

[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]

1055

1056 ^ER = erythema grade; ED = edema grade; h = hour

<b>Extract</b>	<b>Overall Test Group Mean</b>	<b>Overall Control Group Mean</b>	<b>Overall Mean Difference (Test – Control)</b>	<b>Conclusion</b>
SC	0.0	0.0	0.0	Non-Irritant
SO	1.0	0.9	0.1	Non-Irritant

1057

There were no adverse clinical findings or animal deaths; or

1058

The following adverse clinical findings or animal deaths occurred:<sup>38</sup>

<i>Description of adverse clinical findings or animal deaths</i>
--

1059

I confirm that:

1060

The above summary information includes all original and any retest data; and

<sup>38</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1061  I have checked that there are no differences between the complete test report and this  
1062 ASCA Summary Test Report.

1063

1064

1065

1066 \_\_\_\_\_  
Name: [TYPED NAME POSITION]

Date

1067

1068

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1071 **Appendix D: Test Method-Specific ASCA Specifications**  
1072 **and Summary Test Report –MEM Elution Cytotoxicity**  
1073 **(ISO 10993-5)**

1074 **A. ASCA Specifications MEM Elution Cytotoxicity (ISO**  
1075 **10993-5)**

1076 **ISO/IEC 17025 Subclause 6.2(e)**

1077 The procedures, documentation and training program will address the following, at a  
1078 minimum:

- 1079 i. Cell line<sup>39</sup> maintenance (e.g., cell line subculture, cell line storage, storage  
1080 conditions, cell line recovery from storage, use of mycoplasma-free cell line,  
1081 good cell culture practices, morphology assessment),  
1082 ii. Cell counting,  
1083 iii. Cell seeding,  
1084 iv. Addition of test and control samples to the cell cultures,  
1085 v. Scoring of test and control articles including assessment of cellular  
1086 characteristics (e.g., general cell morphology, vacuolization, detachment,  
1087 membrane integrity) and percent lysis,  
1088 vi. Evaluation criteria and basis for retest,  
1089 vii. Data documentation, calculations, analysis and result interpretation (including  
1090 test-specific assessment of borderline results),  
1091 viii. Mock study to assess technician competence in test performance, data  
1092 documentation, and result interpretation (including test-specific assessment of  
1093 borderline results). A mock study protocol should be provided to include the  
1094 following:  
1095  
1096 - test and control articles used,  
1097 - test and control article preparation if this task is conducted by the  
1098 trainee,  
1099 - how test samples and controls are blinded to the trainee,  
1100 - test procedure,  
1101 - how raw data, analysis and result interpretation will be captured by the  
1102 trainee and reviewed by the trainer, and  
1103 - predefined criteria for assessing a trainee's performance in the mock  
1104 study to allow them to begin independent ASCA testing.  
1105  
1106 ix. Minimally, biannual periodic technician proficiency check of negative and  
1107 positive control scoring. The proficiency check procedure should specify the  
1108 following:

---

<sup>39</sup>L929 cell line is recommended for ASCA testing. Other cell lines may be considered if, as part of the ASCA accreditation application, the testing laboratory provides documentation and a justification (i.e., based on a validation report, historical use for FDA submissions) to FDA to support the use of another cell line for MEM elution cytotoxicity testing.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

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- positive and negative controls used,
- how grade 2 and grade 3 results will be generated (e.g., serial dilutions of a positive control),
- evaluation time point(s) used (e.g., 24 hour incubation, 48 hour incubation, and/or 72 hour incubation),
- number of samples and controls used,
- pass/fail criteria. For example, comparison of technician and trainer scores (e.g., percent lysis) and criteria for acceptable level of agreement.

1120

- x. Criteria for technician retraining.

1121

**ISO/IEC 17025 Subclause 7.7(a)**

1123

The testing laboratory agrees that pre-defined criteria for positive/negative/reference control values will be as follows:

1124

1125

1126

1127

1128

1129

- i. each positive control material replicate is  $\geq$  Grade 3,
- ii. each negative control material replicate is Grade 0,
- iii. each vehicle control replicate is Grade 0.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1130 **B. Example ASCA Summary Test Report: MEM Elution**  
1131 **Cytotoxicity (ISO 10993-5)**

1132  
1133 *Note: This example is intended to illustrate the supplemental documentation that would*  
1134 *accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing*  
1135 *laboratory to the device manufacturer.*

1136  
1137 **Administrative Information**

- 1138 1. Testing Laboratory Name:  
1139 2. ASCA Testing Laboratory Identification Number:  
1140 3. Testing Location(s):  
1141 4. Testing Date(s):  
1142 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
1143  Standard (and particular test method) was \*NOT\* in testing laboratory’s scope of  
1144 *ASCA Accreditation*<sup>40</sup>  
1145  Standard (and particular test method) was in testing laboratory’s scope of *ASCA*  
1146 *Accreditation*  
1147  *ASCA Accreditation* was not suspended  
1148  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

1149  
1150 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

- 1151  Test Article was prepared per the above protocol (no deviations/amendments); or  
1152  Test Article was prepared per the above protocol, with the following  
1153 deviations/amendments<sup>41</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

1154 **Test Article:**

- 1155  Entire final finished device  
1156  Representative sample selection per SOP. Included/Excluded components: *[DESCRIBE]*  
1157 \_\_\_\_\_

<sup>40</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

<sup>41</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1158  Other:<sup>42</sup> [DESCRIBE]

1159 **Extraction Solvent:**

1160  MEM with 5-10% animal serum

1161  Other:<sup>43</sup> [DESCRIBE]

1162 **Extraction Ratio:**

1163  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

1164  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*

1165  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

1166  0.2 g/ml (for powder devices)

1167  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
1168 test article as compared to surface/volume ratio) [*Provide information on comparison  
1169 when mass/volume ratio versus surface area/volume ratio is used.*]

1170 \*Note: For absorbent device only: [Specify surface area of test article and the total  
1171 volume of extraction vehicle used taking into account the additional volume from  
1172 absorbency determination.]

1173  Other:<sup>44</sup> [DESCRIBE]

1174 **Extraction Conditions:**

1175  37°C, 24 h

1176  37°C, 72 h

1177  Other:<sup>45</sup> [DESCRIBE]

1178

1179 **Agitation During Extraction:**

1180  Extraction with continuous agitation or circulation

1181  Extraction under static conditions or intermittent agitation<sup>46</sup>: [DESCRIBE and PROVIDE  
1182 JUSTIFICATION]

1183

1184

1185 **Fluid Path Extractions:**

1186  For fluid path devices or components (where fluids contact the channels in the device or  
1187 component, and then the fluid enters the body), the extraction was conducted using protocols  
1188 specific to fluid path, with the following approach:<sup>47</sup>

1189  Complete fill with agitation

1190  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

---

<sup>42</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>43</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>44</sup> *Ibid*

<sup>45</sup> *Ibid*

<sup>46</sup> *Ibid*

<sup>47</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

**Contains Nonbinding Recommendations**

**Draft – Not for Implementation**

- 1191  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]  
 1192  Other: [SUMMARIZE APPROACH]  
 1193

1194 **Extract Observations:**

- 1195  The test article and extract DID NOT change color, and the extract DID NOT appear  
 1196 turbid or have particles.  
 1197  There were changes in color/turbidity or particles in the test article and/or extract OR  
 1198 there was swelling/degradation of the test article.<sup>48</sup>

1199 **ASCA Test Method SOP #:** [ASCACytotox(date/version)]

- 1200  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
 1201 or  
 1202  Test was conducted per the above protocol and 21 CFR 58, with the following  
 1203 deviations/amendments:<sup>49</sup>

*Description of deviations/amendments*

1204  
1205

**Results:**<sup>50</sup>

	<b>24 hour (h) Results (optional)</b>	<b>48 h Results</b>	<b>72 h Results (&gt;48 h device use)</b>	<b>Conclusion</b>
Vehicle Control	Grade 0/0/0	Grade 0/0/0	Grade 0/0/0	Performed as expected
Negative Control: <i>[Specify per SOP]</i>	Grade 0/0/0	Grade 0/0/0	Grade 0/0/0	Performed as expected
Positive Control: <i>[Specify per SOP]</i>	Grade 3/3/3	Grade 4/4/4*	Grade 4/4/4	Performed as expected
Test Article Extract (100% neat)	Grade 0/0/0	Grade 0/0/0	Grade 0/0/0	Non-cytotoxic
<i>[INSERT ROWS FOR ANY ADDITIONAL TEST ARTICLE DILUTION/RETEST DATA]</i>				

<sup>48</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>49</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>50</sup> The complete test report should be included with ASCA Summary Test Report if there were non-zero results for the test article, vehicle control or negative control, or if there were results less than 3 for the positive control at any timepoint.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1206 \*based on prior results (once Grade 4 results are observed, subsequent assessment is not  
1207 necessary for cytotoxicity)

1208

1209

1210 I confirm that:

1211  The above summary information includes all original and any retest data; and

1212  I have checked that there are no differences between the complete test report and this

1213 ASCA Summary Test Report.

---

1214 Name: [TYPED NAME POSITION]

Date

1215

1216

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1217 **Appendix E: Test Method-Specific ASCA Specifications**  
1218 **and Summary Test Report: Dermal Irritation (ISO 10993-**  
1219 **23)**

1220

1221 **A. ASCA Specifications: Dermal Irritation (ISO 10993-**  
1222 **23)**

1223 **ISO/IEC 17025 Subclause 6.2(e)**

1224 The procedures, documentation, and training program will address the following, at a  
1225 minimum:

- 1226 i. Shaving techniques (e.g., to avoid razor burn),  
1227 ii. Application of test samples,  
1228 iii. Representative sample selection (direct contact),  
1229 iv. Differentiation for source of redness (e.g., true irritation versus possible  
1230 irritation from shaving),  
1231 v. Clinical observations (e.g., cage side observation, skin site observation,  
1232 and presence of adverse events), criteria for assessment, data capture, and  
1233 frequency (e.g., minimum daily),  
1234 vi. Evaluation criteria and basis for retest,  
1235 vii. Data documentation, calculations, analysis and result interpretation,  
1236 viii. Minimally, biannual periodic technician proficiency check of positive  
1237 response scoring (in live animals at least once annually). The proficiency  
1238 check procedure should specify, at a minimum, the following:  
1239  
1240 - positive and negative controls used,  
1241 - number of positive and negative controls, number of animals and/or  
1242 number of images used (if images are used for proficiency check),  
1243 - protocol used to conduct the study, and  
1244 - pass/fail criteria. For example, comparison of technician and trainer  
1245 scores and criteria for acceptable level of agreement: for each  
1246 individual site, and the overall score (e.g., irritant vs. non-irritant).  
1247  
1248 ix. Criteria for technician retraining, if needed.

1249  
1250 **ISO/IEC 17025 Subclause 7.7(a)**

1251 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
1252 values will be as follows:

- 1253 i. each sodium chloride and oil control site is Grade 0,  
1254 ii. confirmation of assay sensitivity by running positive control study at least  
1255 every six months or tracking that at least one test article positive result has  
1256 occurred within the previous six months.<sup>51</sup>

---

<sup>51</sup> ISO 10993-23 ISO 10993-23 Biological evaluation of medical devices – Part 23: Tests for skin irritation.

1257

1258

1259

## B. Example ASCA Summary Test Report: Dermal Irritation (ISO 10993-23)

1260

*Note: This example is intended to illustrate the supplemental documentation that would accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing laboratory to the device manufacturer.*

1261

1262

1263

1264

### Administrative Information

1265

1. Testing Laboratory Name:

1266

2. ASCA Testing Laboratory Identification Number:

1267

3. Testing Location(s):

1268

4. Testing Date(s):

1269

5. ASCA Accreditation Status on the Date(s) of Testing:

1270

Standard (and particular test method) was \*NOT\* in testing laboratory's scope of ASCA Accreditation<sup>52</sup>

1271

1272

Standard (and particular test method) was in testing laboratory's scope of ASCA Accreditation

1273

1274

ASCA Accreditation was not suspended

1275

ASCA Accreditation was suspended

*Description of reasons for suspension and their impact on testing results.*

1276

1277

ASCA Test Article Prep SOP#: [ASCATAPrep(date/version)]

1278

Test Article was prepared per the above protocol (no deviations/amendments); or

1279

Test Article was prepared per the above protocol, with the following

1280

deviations/amendments<sup>53</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

1281

**Test Article Type:**

1282

Powder

1283

Solid sample

1284

Test article extracts

<sup>52</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of ASCA Accreditation.

<sup>53</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also request to provide a rationale to support a regulatory decision.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1285  Other<sup>54</sup>: [DESCRIBE]

1286 **Test Article:**

1287  Entire final finished device

1288  Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]  
1289 \_\_\_\_\_

1290  Other:<sup>55</sup> [DESCRIBE]

1291

1292 **Test Article Extraction (if applicable):**

1293 **Extraction Solvent:**

1294  0.9% Sodium Chloride (SC)

1295  Cotton Seed Oil (CSO)/Sesame Oil (SO)

1296  Other:<sup>56</sup> [DESCRIBE]

1297 **Extraction Ratio:**

1298  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

1299  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*

1300  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

1301  0.2 g/ml (for powder devices)

1302  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
1303 test article as compared to surface/volume ratio) [*Provide information on comparison*  
1304 *when mass/volume ratio versus surface area/volume ratio is used.*]

1305 \*Note: For absorbent device only: [*Specify surface area of test article and the total*  
1306 *volume of extraction vehicle used taking into account the additional volume from*  
1307 *absorbency determination.*]

1308  Other:<sup>57</sup> [DESCRIBE]

1309 **Extraction Conditions:**

1310  37°C, 72 h

1311  50°C, 72 h

1312  70°C, 24 h

1313  121°C, 1 h

1314  Other:<sup>58</sup> [DESCRIBE]

1315

1316 **Agitation During Extraction:**

1317  Extraction with continuous agitation or circulation

---

<sup>54</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>55</sup> *Ibid*

<sup>56</sup> *Ibid*

<sup>57</sup> *Ibid*

<sup>58</sup> *Ibid*

***Contains Nonbinding Recommendations***

***Draft – Not for Implementation***

1318  Extraction under static conditions or intermittent agitation<sup>59</sup>: [DESCRIBE and PROVIDE  
1319 JUSTIFICATION]

1320

1321 **Fluid Path Extractions:**

1322  For fluid path devices or components (where fluids contact the channels in the device or  
1323 component, and then the fluid enters the body), the extraction was conducted using protocols  
1324 specific to fluid path, with the following approach:<sup>60</sup>

1325  Complete fill with agitation

1326  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

1327  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]

1328  Other: [SUMMARIZE APPROACH]

1329

1330 **Extract Observations:**

1331  The test article and extract DID NOT change color, and the extract DID NOT appear  
1332 turbid or have particles.

1333  There were changes in color/turbidity or particles in the test article and/or extract OR  
1334 there was swelling/degradation of the test article.<sup>61</sup>

1335

1336 **ASCA Test Method SOP #:** [ASCADermalIrri(date/version)]

1337  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
1338 or

1339  Test was conducted per the above protocol and 21 CFR 58, with the following  
1340 deviations/amendments:<sup>62</sup>

*Description of deviations/amendments*

1341

1342 **Exposure Time**<sup>63</sup>

1343  4 h

1344  4-24 h, specify exposure time: \_\_\_\_\_

1345  Other:<sup>64</sup> [DESCRIBE]

<sup>59</sup> *Ibid*

<sup>60</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

<sup>61</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>62</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>63</sup> This ASCA testing is for limited intact skin contacting medical devices with a single exposure of less than or equal to 24 hours. Other exposure periods (e.g., repeated exposure, single exposure greater than 24 hours) are not included in the scope for ASCA.

<sup>64</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

**Contains Nonbinding Recommendations**

**Draft – Not for Implementation**

1346

1347

**Test Article Application:**

1348

Test article (e.g., powder) is moistened with solvent and applied to the skin: [Describe solvent (e.g., water, saline, oil) used to moisten the test article]

1349

1350

Test article is directedly applied to the skin (i.e., not moistened)

1351

Extracts are applied to the gauze patch and then the gauze patch is applied to the skin (for extract testing)

1352

1353

Other:<sup>65</sup> [DESCRIBE]

1354

1355

**Control Article Application:**

1356

Gauze is moistened with solvent and applied to the skin: [Describe solvent (e.g., water, saline, oil) used to moisten gauze]

1357

1358

Gauze is directedly applied to the skin (i.e., not moistened)

1359

Vehicle control is applied to the gauze patch and then the gauze patch is applied to the skin (for extract testing)

1360

1361

Other:<sup>66</sup> [DESCRIBE]

1362

**Results:<sup>67</sup>**

1363

**Table 1 Summary of Scores for Dermal Irritation\***

Animal Number	Test/Control Article Sites	Score @ 1 hour (h)		Score @ 24h		Score @ 48h		Score @ 72h	
		ER	ED	ER	ED	ER	ED	ER	ED
1	Test Site-1	0	0	0	0	0	0	0	0
	Test Site-2	1	0	0	0	0	0	0	0
	Control Site-1	0	0	0	0	0	0	0	0
	Control Site-2	0	0	0	0	0	0	0	0
2	Test Site-1	0	0	0	0	0	0	0	0
	Test Site-2	0	0	0	0	0	0	0	0
	Control Site-1	0	0	0	0	0	0	0	0
	Control Site-2	0	0	0	0	0	0	0	0
3	Test Site-1	0	0	0	0	0	0	0	0
	Test Site-2	0	0	0	0	0	0	0	0
	Control Site-1	0	0	0	0	0	0	0	0
	Control Site-2	0	0	0	0	0	0	0	0

1364

*[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]*

<sup>65</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>66</sup> *Ibid*

<sup>67</sup> The complete test report should be included with ASCA Summary Test Report if the primary irritation score is calculated using different timepoints besides 24h, 48h and 72h, if there were any non-zero test or control (e.g., direct contact control: gauze; extract test control: sodium chloride or oil) results at any time point.

**Contains Nonbinding Recommendations**

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1365 \*For extract-based tests: animal data<sup>68</sup> for both polar and nonpolar test extracts and  
1366 corresponding vehicle controls should be reported.

1367 ^ER = erythema grade; ED = edema grade

1368 **Table 2 Summary of Primary Irritation Index\***

<b>Animal Number</b>	<b>Test Score Average</b>	<b>-</b>	<b>Control Score Average</b>	<b>Individual Primary Irritation Score</b>				<b>Conclusion</b>
<b>1</b>	0.0	-	0.0					Non-irritant
<b>2</b>	0.0	-	0.0					
<b>3</b>	0.0	-	0.0					

1369 \*For extract-based tests: animal data for both polar and nonpolar test extracts and  
1370 corresponding vehicle controls should be reported.

1371  There were no adverse clinical findings or animal deaths; or

1372  The following adverse clinical findings or animal deaths occurred:<sup>69</sup>

<i>Description of adverse clinical findings or animal deaths</i>
--

1373 I confirm that:

1374  The above summary information includes all original and any retest data; and

1375  I have checked that there are no differences between the complete test report and this  
1376 ASCA Summary Test Report.

1377

1378

1379

1380 Name: [TYPED NAME POSITION]

Date

1381

<sup>68</sup> We support the principles of the “3Rs,” to reduce, refine, and replace animal use in testing when feasible. We encourage sponsors to consult with us if they wish to use a non-animal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method. However, these alternative methods would not be eligible for the ASCA Program. See generally: <https://www.fda.gov/science-research/advancing-regulatory-science/vi-modernizing-safety-testing>

<sup>69</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

1382 **Appendix F: Test Method-Specific ASCA Specifications**  
1383 **and Summary Test Report: Guinea Pig Maximization**  
1384 **Sensitization (ISO 10993-10)**

1385 **A. ASCA Specifications: Guinea Pig Maximization**  
1386 **Sensitization (GPMT) (ISO 10993-10)**

1387  
1388 **ISO/IEC 17025 Subclause 6.2(e)**

1389 The procedures, documentation and training program will address the following, at a  
1390 minimum:

- 1391 i. Shaving techniques (e.g., to avoid razor burn),  
1392 ii. Mixing of extract and adjuvant, including confirmation of homogeneous  
1393 emulsion,  
1394 iii. Intradermal injection techniques (e.g., aseptic technique, correct dosing,  
1395 use of aseptic technique during preparation of the injection site, needle  
1396 size, bevel direction, insertion of the needle parallel with skin surface,  
1397 insertion of the needle at least 2-3 mm into the dermis, formation of skin  
1398 bleb, leaving needle in the injection site for at least 1 second before slowly  
1399 withdrawing needle),  
1400 iv. Intradermal injection techniques for injections containing Freund's  
1401 complete adjuvant (FCA) to minimize local tissue response to FCA (e.g.,  
1402 appropriate injection spacing to enable assessment and avoid coalescence  
1403 of inflammatory lesions, minimization of undesirable dermal side effects  
1404 such as local inflammation and granulomatous reactions at the site of  
1405 injection, skin ulceration, local abscess or tissue sloughing),  
1406 v. Intradermal injection criteria to confirm avoidance of subcutaneous  
1407 injections,  
1408 vi. Sample application to test site,  
1409 vii. Animal wrapping techniques to prevent restriction of animal breathing or  
1410 trauma to the site while maintaining test article exposure,  
1411 viii. Procedure if contact between test or control articles (or patches) and skin  
1412 is interrupted during exposure (e.g., wrap loosens or fall off),  
1413 ix. Differentiation of source of redness during scoring (e.g., true sensitization  
1414 versus mechanical/adhesive irritation),  
1415 x. Minimization of bias during scoring (e.g., without knowledge of the  
1416 treatment),  
1417 xi. Clinical observations (e.g., cage side observation, skin site observation,  
1418 and presence of adverse events), criteria for assessment, data capture, and  
1419 frequency (e.g., minimum daily),

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

- 1420           xii.    Evaluation criteria and basis for pretest, retesting (e.g., invalid control  
1421           results) and rechallenge,<sup>70</sup> if needed,  
1422           xiii.   Data documentation, calculations, analysis, and result interpretation  
1423           (including test-specific assessment of borderline score),  
1424           xiv.    Minimally, quarterly periodic technician proficiency check of positive  
1425           control scoring (in live animals at least once annually). The proficiency  
1426           check procedure should address, at a minimum, the following:  
1427  
1428                   -   positive and negative controls used,  
1429                   -   number of animals used (positive control and negative control) and or  
1430                   -   number of images used (if images are used for proficiency check),  
1431                   -   protocol used to conduct the study,  
1432                   -   evaluation time point(s) used (e.g., 24 hour or 48 hour timepoint or  
1433                   both), and  
1434                   -   pass/fail criteria. For example, comparison of technician and trainer  
1435                   scores and criteria for acceptable level of agreement: for each  
1436                   individual site, and the overall score (e.g., sensitizer vs. non-  
1437                   sensitizer).  
1438  
1439           xv.    Criteria for technician retraining.

1441 **ISO/IEC 17025 Subclause 7.7(a)**

1442 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
1443 values will be as follows:

- 1444           i.    all sodium chloride and oil vehicle control animals have Grade 0 results at all  
1445           sites for all time points,  
1446           ii.   the positive controls are run at least biannually (for each animal source and  
1447           within 3 months of test article test date) and each animal is at least one grade  
1448           higher than concurrently run sodium chloride and oil vehicle controls in at  
1449           least 8 out of 10 positive control animals (for strong sensitizers<sup>71</sup> such as 0.1-  
1450           0.5% dinitrochlorobenzene (DNCB) at induction and 0.05-0.1% DNCB at  
1451           challenge).  
1452

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<sup>70</sup> Per ISO 10993-10:2021 Clause 6.5.6 and Clause 6.6.6, a rechallenge is recommended when the results are equivocal, such as when the test group has a greater number of animals showing a response than the controls but the intensity of the reaction is not greater than that exhibited by the controls. Per Section IV-B 7.7 “Ensuring the validity of results” of this guidance, for GPMT and closed patch sensitization testing, all control sites must have a Grade 0 response; therefore, there should not be equivocal results in which a rechallenge is needed.

<sup>71</sup> The use of a weak to moderate sensitizer (e.g., mercaptobenzothiazole, hexyl cinnamic aldehyde (HCA), benzocaine) as a positive control for ASCA GPMT and closed patch sensitization tests would be considered acceptable if adequate positive control study data are provided as part of the ASCA application demonstrating that appropriate concentrations of the sensitizer are used for the induction and challenge phases to evoke a weak to moderate sensitization response and the response is similar to the sensitization response reported in the literature with similar concentrations of the sensitizer for the induction and challenge phases.



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**B. Example ASCA Summary Test Report: Guinea Pig Maximization Sensitization (ISO 10993-10)**

*Note: This example is intended to illustrate the supplemental documentation that would accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing laboratory to the device manufacturer.*

**Administrative Information**

- 1. Testing Laboratory Name:
- 2. ASCA Testing Laboratory Identification Number:
- 3. Testing Location(s):
- 4. Testing Date(s):
- 5. *ASCA Accreditation* Status on the Date(s) of Testing:
  - Standard (and particular test method) was \*NOT\* in testing laboratory’s scope of *ASCA Accreditation*<sup>72</sup>
  - Standard (and particular test method) was in testing laboratory’s scope of *ASCA Accreditation*
    - ASCA Accreditation* was not suspended
    - ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

**ASCA Test Article Prep SOP#:** [ASCATAPrep(date/version)]

- Test Article was prepared per the above protocol (no deviations/amendments); or
- Test Article was prepared per the above protocol, with the following deviations/amendments<sup>73</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

**Test Article:**

- Entire final finished device
- Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]

<sup>72</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

<sup>73</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

*Contains Nonbinding Recommendations*

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1481  Other:<sup>74</sup> [DESCRIBE]

1482 **Extraction Solvent:**

- 1483  0.9% Sodium Chloride (SC)  
1484  Cotton Seed Oil (CSO)/Sesame Oil (SO)  
1485  Other:<sup>75</sup> [DESCRIBE]

1486 **Extraction Ratio:**

- 1487  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*  
1488  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*  
1489  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*  
1490  0.2 g/ml (for powder devices)  
1491  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
1492 test article as compared to surface/volume ratio) [*Provide information on comparison*  
1493 *when mass/volume ratio versus surface area/volume ratio is used.*]  
1494 \*Note: For absorbent device only: [*Specify surface area of test article and the total*  
1495 *volume of extraction vehicle used taking into account the additional volume from*  
1496 *absorbency determination.*]  
1497  Other:<sup>76</sup> [DESCRIBE]

1498 **Extraction Conditions:**

- 1499  37°C, 72 h  
1500  50°C, 72 h  
1501  70°C, 24 h  
1502  121°C, 1 h  
1503  Other:<sup>77</sup> [DESCRIBE]

1504

1505 **Agitation During Extraction:**

- 1506  Extraction with continuous agitation or circulation  
1507  Extraction under static conditions or intermittent agitation<sup>78</sup>: [DESCRIBE and PROVIDE  
1508 JUSTIFICATION]

1509

1510 **Fluid Path Extractions:**

- 1511  For fluid path devices or components (where fluids contact the channels in the device or  
1512 component, and then the fluid enters the body), the extraction was conducted using protocols  
1513 specific to fluid path, with the following approach:<sup>79</sup>

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<sup>74</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>75</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also request to provide a rationale to support a regulatory decision.

<sup>76</sup> *Ibid*

<sup>77</sup> *Ibid*

<sup>78</sup> *Ibid*

<sup>79</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

***Contains Nonbinding Recommendations***

***Draft – Not for Implementation***

- 1514  Complete fill with agitation  
 1515  Partial fill with agitation (ISO 10993-12 surface/volume ratio)  
 1516  Partial fill with agitation (other surface/volume ratio): [*DESCRIBE RATIO USED*]  
 1517  Other: [*SUMMARIZE APPROACH*]

1518

1519 **Extract Observations:**

1520  The test article and extract DID NOT change color, and the extract DID NOT appear  
 1521 turbid or have particles.

1522  There were changes in color/turbidity or particles in the test article and/or extract OR  
 1523 there was swelling/degradation of the test article.<sup>80</sup> **ASCA Test Method SOP #:**  
 1524 [*ASCAMaximizationSensi(date/version)*]

1525  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
 1526 or

1527  Test was conducted per the above protocol and 21 CFR 58, with the following  
 1528 deviations/amendments:<sup>81</sup>

*Description of deviations/amendments*

1529 **Results:**<sup>82</sup>

1530

**Table 1 Summary of Scores for Sensitization**

Group	Animal Number	24 hours (h)		48h		Sensitization Frequency	Conclusion
		Control Site	Test Site	Control Site	Test Site		
SC Test	1	0	0	0	0	0%	Non-sensitizer
	2	0	0	0	0		
	3	0	0	0	0		
	4	0	0	0	0		
	5	0	0	0	0		
	6	0	0	0	0		
	7	0	0	0	0		
	8	0	0	0	0		
	9	0	0	0	0		

<sup>80</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>81</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>82</sup> The complete test report should be included with ASCA Summary Test Report, if the Magnusson and Kligman grades of 1 or greater are observed in the test or the sodium chloride and oil vehicle control groups (i.e., per ISO 10993-10:2021, Clause 6.5.6).

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	<b>10</b>	0	0	0	0		
<b>SC Control</b>	<b>1</b>	0	0	0	0	0%	Performed as expected
	<b>2</b>	0	0	0	0		
	<b>3</b>	0	0	0	0		
	<b>4</b>	0	0	0	0		
	<b>5</b>	0	0	0	0		
<b>SO Test</b>	<b>1</b>	0	0	0	0	0%	Non-sensitizer
	<b>2</b>	0	0	0	0		
	<b>3</b>	0	0	0	0		
	<b>4</b>	0	0	0	0		
	<b>5</b>	0	0	0	0		
	<b>6</b>	0	0	0	0		
	<b>7</b>	0	0	0	0		
	<b>8</b>	0	0	0	0		
	<b>9</b>	0	0	0	0		
	<b>10</b>	0	0	0	0		
<b>SO Control</b>	<b>1</b>	0	0	0	0	0%	Performed as expected
	<b>2</b>	0	0	0	0		
	<b>3</b>	0	0	0	0		
	<b>4</b>	0	0	0	0		
	<b>5</b>	0	0	0	0		
<b>Positive Control* [Specify]</b>	<b>1</b>	0	2	0	2	100%	Performed as expected
	<b>2</b>	0	2	0	1		
	<b>3</b>	0	2	0	3		
	<b>4</b>	0	2	0	2		
	<b>5</b>	0	2	0	2		

[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA, AND FOR PERIODIC CONTROL TESTING]

1531  
1532

1533 **\*Periodic/concurrent positive control study**

- 1534  Positive control induction I concentration and solvent: [DESCRIBE]
- 1535  Positive control induction II concentration and solvent: [DESCRIBE]
- 1536  Positive control challenge concentration and solvent: [DESCRIBE]
- 1537  Vehicle control for periodic positive control study: [DESCRIBE]
- 1538  The same source, strain, and treatment methods used for positive control testing and done
- 1539 within 3 months of (i.e., before or after) test article test date
- 1540  The following differences in source, strain, treatment methods or timing of the positive
- 1541 control occurred:<sup>83</sup>

<sup>83</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

*Description of differences in source, strain, treatment methods, or timing of the positive control.*

1542

- 1543  There were no adverse clinical findings or animal deaths; or  
1544  The following adverse clinical findings or animal deaths occurred: <sup>84</sup>

*Description of adverse clinical findings or animal deaths.*

1545 I confirm that:

- 1546  The above summary information includes all original and any retest data; and  
1547  I have checked that there are no differences between the complete test report and this  
1548 ASCA Summary Test Report.

1549

1550

1551

1552 Name: [TYPED NAME POSITION]

Date

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<sup>84</sup> *Ibid*

1553 **Appendix G: Test Method-Specific ASCA Specifications**  
1554 **and Summary Test Report: Closed Patch Sensitization**  
1555 **(ISO 10993-10)**

1556

1557 **A. ASCA Specifications: Closed Patch Sensitization (ISO**  
1558 **10993-10)**

1559

1560 **ISO/IEC 17025 Subclause 6.2(e)**

1561 The procedures, documentation and training program will address the following, at a  
1562 minimum:

- 1563 i. Shaving techniques (e.g., to avoid razor burn),  
1564 ii. Representative sample selection (for direct contact test),  
1565 iii. Sample application to test site,  
1566 iv. Animal wrapping techniques to prevent restriction of animal breathing or  
1567 trauma to the site while maintaining test article exposure,  
1568 v. Procedure if contact between test or control articles (or patches) and skin  
1569 is interrupted during exposure (e.g., wrap loosens or fall off),  
1570 vi. Differentiation of source of redness during scoring (e.g., true sensitization  
1571 versus mechanical/adhesive irritation),  
1572 vii. Minimization of bias during scoring (e.g., without knowledge of the  
1573 treatment),  
1574 viii. Clinical observations (e.g., cage side observation, skin site observation,  
1575 and presence of adverse events), criteria for assessment, data capture, and  
1576 frequency (e.g., minimum daily),  
1577 ix. Evaluation criteria and basis for pretest, retesting (e.g., invalid control  
1578 results) and rechallenge<sup>85</sup>, if needed,  
1579 x. Data documentation, calculations, analysis and result interpretation  
1580 (including test-specific assessment of borderline score),  
1581 xi. Minimally, quarterly periodic technician proficiency check of positive  
1582 control scoring (in live animals at least once annually). The proficiency  
1583 check procedure should address, at a minimum, the following:  
1584  
1585 - positive and negative controls used,  
1586 - number of animals used (positive control and negative control) and or  
1587 number of images used (if images are used for proficiency check),  
1588 - protocol used to conduct the study,

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<sup>85</sup> Per ISO 10993-10:2021 Clause 6.5.6 and Clause 6.6.6, a rechallenge is recommended when the results are equivocal, such as when the test group has a greater number of animals showing a response than the controls but the intensity of the reaction is not greater than that exhibited by the controls. Per Section IV-B 7.7 “Ensuring the validity of results” of this guidance, for GPMT and closed patch sensitization testing, all control sites must have a Grade 0 response; therefore, there should not be equivocal results in which a rechallenge is needed.

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- 1589 - evaluation time point(s) used (e.g., 24 hour or 48 hour timepoint or  
1590 both), and  
1591 - pass/fail criteria. For example, comparison of technician and trainer  
1592 scores and criteria for acceptable level of agreement: for each  
1593 individual site, and the overall score (e.g., sensitizer vs. non-  
1594 sensitizer).  
1595  
1596 xii. Criteria for technician retraining.  
1597  
1598

1599 **ISO/IEC 17025 Subclause 7.7(a)**

1600 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
1601 values will be as follows:

- 1602 i. all negative control animals (e.g., sodium chloride or oil vehicles or negative  
1603 control materials) have Grade 0 results at all sites for all time points,  
1604 ii. the positive controls are run at least biannually (for each animal source and  
1605 within 3 months of test article test date) and each animal is at least one Grade  
1606 higher than concurrently run sodium chloride and oil vehicle controls in at  
1607 least 8 out of 10 positive control animals (for strong sensitizers<sup>86</sup> such as 0.1-  
1608 0.5% DNCB at induction and 0.05-0.1% DNCB at challenge).  
1609

1610 **B. Example ASCA Summary Test Report: Closed Patch**  
1611 **Sensitization (ISO 10993-10)**

1612  
1613 *Note: This example is intended to illustrate the supplemental documentation that would*  
1614 *accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing*  
1615 *laboratory to the device manufacturer.*  
1616

1617 **Administrative Information**

- 1618 1. Testing Laboratory Name:  
1619 2. ASCA Testing Laboratory Identification Number:  
1620 3. Testing Location(s):  
1621 4. Testing Date(s):  
1622 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
1623  Standard (and particular test method) was \*NOT\* in testing laboratory's scope of  
1624 *ASCA Accreditation*<sup>87</sup>

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<sup>86</sup> Per ISO 10993-10:2021 Clause 6.5.6 and Clause 6.6.6, a rechallenge is recommended when the results are equivocal, such as when the test group has a greater number of animals showing a response than the controls but the intensity of the reaction is not greater than that exhibited by the controls. Per Section IV-B 7.7 “Ensuring the validity of results” of this guidance, for GPMT and closed patch sensitization testing, all control sites must have a Grade 0 response; therefore, there should not be equivocal results in which a rechallenge is needed.

<sup>87</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1625  Standard (and particular test method) was in testing laboratory's scope of *ASCA*  
1626 *Accreditation*

1627  *ASCA Accreditation* was not suspended

1628  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

1629

1630 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

1631  Test Article was prepared per the above protocol (no deviations/amendments); or

1632  Test Article was prepared per the above protocol, with the following  
1633 deviations/amendments<sup>88</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

1634

1635 **Test Article Type:**

1636  Powder

1637  Solid sample

1638  Test article extracts

1639  Other:<sup>89</sup> *[DESCRIBE]*

1640 **Test Article:**

1641  Entire final finished device

1642  Representative sample selection per SOP. Included/Excluded components: *[DESCRIBE]*

1643

1644  Other:<sup>90</sup> *[DESCRIBE]*

1645

1646 **Test Article Extraction (if applicable):**

1647 **Extraction Solvent:**

1648  0.9% Sodium Chloride (SC)

1649  Cotton Seed Oil (CSO)/Sesame Oil (SO)

1650  Other:<sup>91</sup> *[DESCRIBE]*

1651 **Extraction Ratio:**

1652  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

<sup>88</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>89</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>90</sup> *Ibid*

<sup>91</sup> *Ibid*



*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

- 1653  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*  
1654  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*  
1655  0.2 g/ml (for powder devices)  
1656  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
1657 test article as compared to surface/volume ratio) [*Provide information on comparison*  
1658 *when mass/volume ratio versus surface area/volume ratio is used.*]  
1659 \*Note: For absorbent device only: [*Specify surface area of test article and the total*  
1660 *volume of extraction vehicle used taking into account the additional volume from*  
1661 *absorbency determination.*]

1662  Other:<sup>92</sup> [DESCRIBE]

1663 **Extraction Conditions:**

- 1664  37°C, 72 h  
1665  50°C, 72 h  
1666  70°C, 24 h  
1667  121°C, 1 h  
1668  Other:<sup>93</sup> [DESCRIBE]

1669

1670 **Agitation During Extraction:**

- 1671  Extraction with continuous agitation or circulation  
1672  Extraction under static conditions or intermittent agitation<sup>94</sup>: [DESCRIBE and PROVIDE  
1673 JUSTIFICATION]

1674

1675 **Fluid Path Extractions:**

- 1676  For fluid path devices or components (where fluids contact the channels in the device or  
1677 component, and then the fluid enters the body), the extraction was conducted using protocols  
1678 specific to fluid path, with the following approach:<sup>95</sup>  
1679  Complete fill with agitation  
1680  Partial fill with agitation (ISO 10993-12 surface/volume ratio)  
1681  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]  
1682  Other: [SUMMARIZE APPROACH]

1683

1684 **Extract Observations:**

- 1685  The test article and extract DID NOT change color, and the extract DID NOT appear  
1686 turbid or have particles.

---

<sup>92</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>93</sup> *Ibid*

<sup>94</sup> *Ibid*

<sup>95</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

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1687  There were changes in color/turbidity or particles in the test article and/or extract OR  
1688 there was swelling/degradation of the test article.<sup>96</sup>

1689

1690 **ASCA Test Method SOP #:** [ASCAPatchSens(date/version)]

1691  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
1692 or

1693  Test was conducted per the above protocol and 21 CFR 58, with the following  
1694 deviations/amendments:<sup>97</sup>

*Description of deviations/amendments*

1695

1696 **Test Article Application:**

1697  Test article (e.g., powder) is moistened with solvent and applied to the skin: [Describe  
1698 solvent (e.g., water, saline, oil) used to moisten the test article]

1699  Test article is directedly applied to the skin (i.e., not moistened)

1700  Extracts are applied to the patch (filter paper or absorbent gauze) and then the patch is  
1701 applied to the skin (for extract testing)

1702  Other:<sup>98</sup> [DESCRIBE]

1703

1704 **Control Article Application:**

1705  Gauze is moistened with solvent and applied to the skin: [Describe solvent (e.g., water,  
1706 saline, oil) used to moisten gauze]

1707  Gauze is directedly applied to the skin (i.e., not moistened)

1708  Vehicle control is applied to the gauze patch and then the gauze patch is applied to the  
1709 skin (for extract testing)

1710  Other:<sup>99</sup> [DESCRIBE]

1711

1712 **Results:**<sup>100</sup>

<sup>96</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>97</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>98</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>99</sup> *Ibid*

<sup>100</sup> The complete test report should be included with ASCA Summary Test Report if the Magnusson and Kligman grades of 1 or greater observed in the test group, provided grades of less than 1 are seen in negative control animals (i.e., per ISO 10993-10:2021, Clause 6.6.6), or the sodium chloride and oil vehicle controls are > Grade 0.

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1713

**Table 1 Summary of Scores for Sensitization\***

Group	Animal Number	24 hours (h)		48h		Sensitization Frequency	Conclusion
		Control Site	Test Site	Control Site	Test Site		
<b>Test</b>	<b>1</b>	0	0	0	0	0%	Non-sensitizer
	<b>2</b>	0	0	0	0		
	<b>3</b>	0	0	0	0		
	<b>4</b>	0	0	0	0		
	<b>5</b>	0	0	0	0		
	<b>6</b>	0	0	0	0		
	<b>7</b>	0	0	0	0		
	<b>8</b>	0	0	0	0		
	<b>9</b>	0	0	0	0		
	<b>10</b>	0	0	0	0		
<b>Negative Control: [Specify]</b>	<b>1</b>	0	0	0	0	0%	Performed as expected
	<b>2</b>	0	0	0	0		
	<b>3</b>	0	0	0	0		
	<b>4</b>	0	0	0	0		
	<b>5</b>	0	0	0	0		
<b>Concurrent Positive Control: Positive Control* [Specify]</b>	<b>1</b>	0	2	0	2	100%	Performed as expected
	<b>2</b>	0	2	0	1		
	<b>3</b>	0	2	0	3		
	<b>4</b>	0	2	0	2		
	<b>5</b>	0	2	0	2		

1714  
1715

*[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA AND FOR PERIODIC CONTROL TESTING]*

1716  
1717

*\*For extract-based tests: animal data for both polar and nonpolar test extracts and corresponding vehicle controls should be reported.*

1718

**\*\*Periodic/concurrent positive control study**

1719  
1720  
1721  
1722

- Positive control induction I concentration and solvent: *[DESCRIBE]*
- Positive control induction II concentration and solvent: *[DESCRIBE]*
- Positive control challenge concentration and solvent: *[DESCRIBE]*
- Vehicle control for periodic positive control study: *[DESCRIBE]*

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- 1723  The same source, strain, and treatment methods used for positive control testing and done  
1724 within 3 months of (i.e., before or after) test article test date  
1725  The following differences in source, strain, treatment methods or timing of the positive  
1726 control occurred:<sup>101</sup>

*Description of differences in source, strain, treatment methods, or timing of the positive control*

- 1727  
1728  There were no adverse clinical findings or animal deaths; or  
1729  The following adverse clinical findings or animal deaths occurred:<sup>102</sup>

*Description of adverse clinical findings or animal deaths*

1730 I confirm that:

- 1731  The above summary information includes all original and any retest data; and  
1732  I have checked that there are no differences between the complete test report and this  
1733 ASCA Summary Test Report.

1734  
1735  
1736  
1737

1738 Name: [TYPED NAME POSITION]

Date

1739  
1740

<sup>101</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>102</sup> *Ibid*

1741 **Appendix H: Test Method-Specific ASCA Specifications**  
1742 **and Summary Test Report: Acute Systemic Toxicity (ISO**  
1743 **10993-11)**

1744

1745 **A. ASCA Specifications: Acute Systemic Toxicity (ISO**  
1746 **10993-11)**

1747

1748 **ISO/IEC 17025 Subclause 6.2(e)**

1749 The procedures, documentation and training program will address the following, at a  
1750 minimum:

- 1751 i. Balance use and calibration to ensure appropriate sensitivity,  
1752 ii. Weight range of animals,  
1753 iii. Temperature for test sample (e.g., room or body temperature) to be  
1754 delivered to the animals,  
1755 iv. Intraperitoneal (IP) and intravenous (IV) injection techniques and signs to  
1756 confirm appropriate injection location,  
1757 v. Injection rate (e.g., not to exceed 2 ml/min) and how the specified  
1758 injection rate is achieved,  
1759 vi. Evaluation criteria and basis for retest,  
1760 vii. Data documentation, calculations, analysis and result interpretation,  
1761 viii. Clinical observations by performing a cage-side observation for overt  
1762 clinical signs using common laboratory descriptors of clinical effects per  
1763 ASTM F750 and for any mortality along with recording of data, and  
1764 timing of observations  
1765 ix. Minimally, technician proficiency check on injection techniques prior to  
1766 conduct of next test if it has been more than one month between technician  
1767 conduct of a study. The proficiency check procedure should specify the  
1768 following:  
1769  
1770 - restraining technique,  
1771 - injection solutions used (for IV and IP injections),  
1772 - number of animals used for proficiency check on injection (for IV and  
1773 IP), and  
1774 - pass/fail criteria. For example, acceptable level of successful injection.  
1775 Acceptance criteria for successful injection needs to be clearly defined  
1776 and including details on:  
1777  
1778 o ensuring injection rate 2mL/min is achieved,  
1779 o ensuring complete volume of liquid is injected,  
1780 o method to ensure that the needle is in the vein for IV  
1781 injection, and

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1782 ○ method or technique used to confirm that vital organs are  
1783 not punctured or injured during the IP injection.

1784  
1785 x. Criteria for technician retraining.

1786  
1787 **ISO/IEC 17025 Subclause 7.7(a)**

1788 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
1789 values will be as follows:

1790 i. all sodium chloride and oil control animals result in no adverse clinical  
1791 findings, no decrease in body weight > 10% per animal, and no deaths.

1792

1793 **B. Example ASCA Summary Test Report: Acute Systemic**  
1794 **Toxicity (ISO 10993-11)**

1795

1796 *Note: This example is intended to illustrate the supplemental documentation that would*  
1797 *accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing*  
1798 *laboratory to the device manufacturer.*

1799

1800 **Administrative Information**

1801 1. Testing Laboratory Name:

1802 2. ASCA Testing Laboratory Identification Number:

1803 3. Testing Location(s):

1804 4. Testing Date(s):

1805 5. *ASCA Accreditation* Status on the Date(s) of Testing:

1806  Standard (and particular test method) was \*NOT\* in testing laboratory’s scope of  
1807 *ASCA Accreditation*<sup>103</sup>

1808  Standard (and particular test method) was in testing laboratory’s scope of *ASCA*  
1809 *Accreditation*

1810  *ASCA Accreditation* was not suspended

1811  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

1812

1813 **ASCA Test Article Prep SOP#: [ASCATAPrep(date/version)]**

1814  Test Article was prepared per the above protocol (no deviations/amendments); or

<sup>103</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

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1815  Test Article was prepared per the above protocol, with the following  
1816 deviations/amendments<sup>104</sup> (e.g., filtering, extract manipulation, pH adjustment):

<i>Description of deviations/amendments</i>
---

1817 **Test Article:**

1818  Entire final finished device

1819  Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]

1820 \_\_\_\_\_

1821  Other:<sup>105</sup> [DESCRIBE]

1822 **Extraction Solvent:**

1823  0.9% Sodium Chloride (SC)

1824  Cotton Seed Oil (CSO)/Sesame Oil (SO)

1825  Other:<sup>106</sup> [DESCRIBE]

1826 **Extraction Ratio:**

1827  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

1828  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*

1829  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

1830  0.2 g/ml (for powder devices)

1831  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
1832 test article as compared to surface/volume ratio) [*Provide information on comparison*  
1833 *when mass/volume ratio versus surface area/volume ratio is used.*]

1834 \*Note: For absorbent device only: [Specify surface area of test article and the total  
1835 volume of extraction vehicle used taking into account the additional volume from  
1836 absorbency determination.]

1837  Other:<sup>107</sup> [DESCRIBE]

1838 **Extraction Conditions:**

1839  37°C, 72 h

1840  50°C, 72 h

1841  70°C, 24 h

1842  121°C, 1 h

1843  Other:<sup>108</sup> [DESCRIBE]

1844

<sup>104</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>105</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>106</sup> *Ibid*

<sup>107</sup> *Ibid*

<sup>108</sup> *Ibid*

**Contains Nonbinding Recommendations**

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1845 **Agitation During Extraction:**

- 1846  Extraction with continuous agitation or circulation  
1847  Extraction under static conditions or intermittent agitation<sup>109</sup>: [DESCRIBE and PROVIDE  
1848 JUSTIFICATION]\_\_\_\_\_

1849  
1850 **Fluid Path Extractions:**

- 1851  For fluid path devices or components (where fluids contact the channels in the device or  
1852 component, and then the fluid enters the body), the extraction was conducted using protocols  
1853 specific to fluid path, with the following approach:<sup>110</sup>  
1854  Complete fill with agitation  
1855  Partial fill with agitation (ISO 10993-12 surface/volume ratio)  
1856  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]  
1857  Other: [SUMMARIZE APPROACH]

1858 **Extract Observations:**

- 1859  The test article and extract DID NOT change color, and the extract DID NOT appear  
1860 turbid or have particles.  
1861  There were changes in color/turbidity or particles in the test article and/or extract OR  
1862 there was swelling/degradation of the test article.<sup>111</sup>

1863 **ASCA Test Method SOP #:** [ASCAAcuteTox(date/version)]\_\_\_\_\_

- 1864  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
1865 or  
1866  Test was conducted per the above protocol and 21 CFR 58, with the following  
1867 deviations/amendments:<sup>112</sup>

*Description of deviations/amendments*

1868 **Results:**<sup>113</sup>

---

<sup>109</sup>In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>110</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

<sup>111</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>112</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>113</sup> The complete test report should be included with ASCA Summary Test Report if controls did not perform as expected, any animals were found dead or were euthanized, behavior such as convulsions or prostration occurred in any animals, or a body weight loss greater than 10 % occurred in any animals.



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1869

**Table 1 Summary of Test Results<sup>114</sup>**

Extract	Animal Number	Body Weight (g)				Weight Change	Conclusion (Based on Body Weight and Clinical Findings)
		Day 0	Day 1	Day 2	Day 3		
<b>SC Test</b>	<b>1</b>	20.4	20.8	21.1	21.7	1.3	No acute systemic toxicity
	<b>2</b>	19.6	20.4	20.3	21.7	2.1	No acute systemic toxicity
	<b>3</b>	19.6	19.9	20.1	20.7	1.1	No acute systemic toxicity
	<b>4</b>	20.4	19.8	20.3	21.1	0.7	No acute systemic toxicity
	<b>5</b>	17.9	18.6	19.0	19.7	1.8	No acute systemic toxicity
<b>SC Control</b>	<b>1</b>	17.9	19.9	19.8	20.4	2.5	Performed as expected
	<b>2</b>	19.8	20.0	20.9	22.3	2.5	Performed as expected
	<b>3</b>	19.9	20.3	20.8	21.4	1.5	Performed as expected
	<b>4</b>	17.9	17.8	17.9	18.6	0.7	Performed as expected
	<b>5</b>	22.1	22.9	23.1	24.3	2.2	Performed as expected
<b>SO Test</b>	<b>1</b>	22.2	22.9	22.8	23.4	1.2	Not systemically toxic
	<b>2</b>	20.2	21.3	21.4	21.8	1.6	Not systemically toxic
	<b>3</b>	19.0	19.2	19.3	20.2	1.2	Not systemically toxic
	<b>4</b>	18.5	19.8	20.5	21.6	3.1	Not systemically toxic
	<b>5</b>	19.4	20.2	19.8	20.0	0.6	Not systemically toxic
<b>SO Control</b>	<b>1</b>	19.7	20.2	20.5	21.9	2.2	Not systemically toxic
	<b>2</b>	19.4	19.9	19.7	20.0	0.6	Not systemically toxic
	<b>3</b>	21.2	21.7	22.2	23.6	2.4	Not systemically toxic
	<b>4</b>	20.9	21.7	22.0	23.1	2.2	Not systemically toxic
	<b>5</b>	20.3	21.1	21.6	23.4	3.1	Not systemically toxic

1870

*[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]*

1871  There were no adverse clinical findings or animal deaths; or

1872  The following adverse clinical findings or animal deaths occurred:<sup>115</sup>

<i>Description of adverse clinical findings or animal deaths</i>
--

1873 I confirm that:

1874  The above summary information includes all original and any retest data; and

1875  I have checked that there are no differences between the complete test report and this

1876 ASCA Summary Test Report.

1877

1878

1879

<sup>114</sup> This is an example of how data from an acute systemic toxicity test could be presented.

<sup>115</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

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1880 Name: [TYPED NAME POSITION]

Date

1881

1882

1883

1884

DRAFT

1885 **Appendix I: Test Method-Specific ASCA Specifications**  
1886 **and Summary Test Report: Material-Mediated**  
1887 **Pyrogenicity (ISO 10993-11 and USP 151)**  
1888

1889 **A. ASCA Specifications: Material-Mediated Pyrogenicity**  
1890 **(ISO 10993-11 and USP 151)**

1891  
1892 **ISO/IEC 17025 Subclause 6.2(e)**

1893 The procedures, documentation, and training program will address the following, at a  
1894 minimum:

- 1895 i. Use of “pyrogen-free” /depyrogenated glassware and “pyrogen-free”<sup>116</sup>  
1896 saline (e.g., USP Sodium Chloride for Injection) for extraction,  
1897 ii. Temperature probe use and calibration to ensure appropriate sensitivity,  
1898 iii. Noise level maintenance to ensure animals are housed in a quiet  
1899 environment for testing,  
1900 iv. Sham test procedure,  
1901 v. Animal restraining and transfer,  
1902 vi. Placement of temperature-sensing probe (depth and duration),  
1903 vii. Animal temperature monitoring techniques (e.g., continuous monitoring  
1904 with instrumentation),  
1905 viii. Temperature for test sample (i.e., body temperature) to be delivered to the  
1906 animals,  
1907 ix. Intravenous (IV) injection techniques and signs to confirm appropriate  
1908 injection location (e.g., marginal ear vein) including immediate outcomes,  
1909 descriptions of observations to ensure that injection was successfully  
1910 administered within 10 minutes,  
1911 x. Evaluation criteria and basis for retest,  
1912 xi. Data documentation, calculations, analysis and result interpretation,  
1913 xii. Criteria for reusing rabbits across studies (e.g., between different studies  
1914 and over an animal’s life), and  
1915 xiii. Criteria for technician retraining.

1916  
1917  
1918 **ISO/IEC 17025 Subclause 7.7(a)**

1919 For material-mediated pyrogenicity testing there are no predefined criteria for  
1920 positive/negative/reference control values.

---

<sup>116</sup> The use of the phrase “pyrogen-free” here is intended to refer to a common term used by reagent suppliers for sodium chloride for injection that meets USP monograph. FDA does not recommend use of the term pyrogen-free in the labeling of devices, unless the complete removal of pyrogens can be established. See FDA’s guidance “Submission and Review of Sterility Information in Premarket Notification (510(k)) Submissions for Devices Labeled as Sterile” available at <https://www.fda.gov/media/74445/download>

1921

1922

## B. Example ASCA Summary Test Report: Material-Mediated Pyrogenicity (ISO 10993-11 and USP 151)

1923

1924

1925

*Note: This example is intended to illustrate the supplemental documentation that would accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing laboratory to the device manufacturer.*

1926

1927

1928

1929

### Administrative Information

1930

1. Testing Laboratory Name:

1931

2. ASCA Testing Laboratory Identification Number:

1932

3. Testing Location(s):

1933

4. Testing Date(s):

1934

5. ASCA Accreditation Status on the Date(s) of Testing:

1935

Standard (and particular test method) was \*NOT\* in testing laboratory's scope of ASCA Accreditation<sup>117</sup>

1936

1937

Standard (and particular test method) was in testing laboratory's scope of ASCA Accreditation

1938

1939

ASCA Accreditation was not suspended

1940

ASCA Accreditation was suspended

*Description of reasons for suspension and their impact on testing results.*

1941

1942

ASCA Test Article Prep SOP#: [ASCATAPrep(date/version)]

1943

Test Article was prepared per the above protocol (no deviations/amendments); or

1944

Test Article was prepared per the above protocol, with the following

1945

deviations/amendments<sup>118</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

1946

### Test Article:

1947

Entire final finished device

1948

Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]

<sup>117</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of ASCA Accreditation.

<sup>118</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

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1949  Other:<sup>119</sup> [DESCRIBE]

1950 **Extraction Solvent:**

1951  0.9% Sterile “Pyrogen-Free”<sup>120</sup> Saline

1952  Other:<sup>121</sup> [DESCRIBE]

1953 **Extraction Ratio:**

1954  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

1955  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)\*

1956  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

1957  0.2 g/ml (for powder devices)

1958  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more

1959 test article as compared to surface/volume ratio) [*Provide information on comparison*

1960 *when mass/volume ratio versus surface area/volume ratio is used.*]

1961 \*Note: For absorbent device only: [*Specify surface area of test article and the total*

1962 *volume of extraction vehicle used taking into account the additional volume from*

1963 *absorbency determination.*]

1964  Other:<sup>122</sup> [DESCRIBE]

1965 **Extraction Conditions:**

1966  37°C, 72 h

1967  50°C, 72 h

1968  70°C, 24 h

1969  121°C, 1 h

1970  Other:<sup>123</sup> [DESCRIBE]

1971

1972 **Agitation During Extraction:**

1973  Extraction with continuous agitation or circulation

1974  Extraction under static conditions or intermittent agitation<sup>124</sup>: [DESCRIBE and PROVIDE

1975 JUSTIFICATION]

1976

1977

---

<sup>119</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>120</sup> The use of the phrase “pyrogen-free” here is intended to refer to a common term used by reagent suppliers for sodium chloride for injection that meets USP monograph. FDA does not recommend use of the term pyrogen-free in the labeling of devices, unless the complete removal of pyrogens can be established. See FDA’s guidance “Submission and Review of Sterility Information in Premarket Notification (510(k)) Submissions for Devices Labeled as Sterile” available at <https://www.fda.gov/media/74445/download>

<sup>121</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>122</sup> *Ibid*

<sup>123</sup> *Ibid*

<sup>124</sup> *Ibid*

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

1978 **Fluid Path Extractions:**

1979  For fluid path devices or components (where fluids contact the channels in the device or  
1980 component, and then the fluid enters the body), the extraction was conducted using protocols  
1981 specific to fluid path, with the following approach:<sup>125</sup>

- 1982  Complete fill with agitation  
1983  Partial fill with agitation (ISO 10993-12 surface/volume ratio)  
1984  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]  
1985  Other: [SUMMARIZE APPROACH]  
1986

1987 **Extract Observations:**

1988  The test article and extract DID NOT change color, and the extract DID NOT appear  
1989 turbid or have particles.

1990  There were changes in color/ turbidity or particles in the test article and/or extract OR  
1991 there was swelling/degradation of the test article.<sup>126</sup>  
1992

1993 **ASCA Test Method SOP #:** [ASCAPyrogenicity(date/version)]

- 1994  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
1995 or  
1996  Test was conducted per the above protocol and 21 CFR 58, with the following  
1997 deviations/amendments:<sup>127</sup>

<i>Description of deviations/amendments</i>
---

1998 **Results:**<sup>128</sup>

1999 **Table 1 Pyrogen Test Data**<sup>129</sup>

---

<sup>125</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

<sup>126</sup> In this situation, the complete test report should be included with ASCA Summary Test Report.. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>127</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report.. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>128</sup> The complete test report should be included with ASCA Summary Test Report if any rabbit has a baseline temperature exceeding 39.8°C or if any rabbit has a temperature rise  $\geq 0.5^\circ\text{C}$ .

<sup>129</sup> This is an example of how data from a material-mediated pyrogenicity test could be presented.

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<b>Animal Number</b>	<b>Baseline Temp (°C)</b>	<b>1.0 hour (h) Temp (°C)</b>	<b>1.5 h Temp (°C)</b>	<b>2.0 h Temp (°C)</b>	<b>2.5 h Temp (°C)</b>	<b>3.0 h Temp (°C)</b>	<b>Temp Increase (°C)</b>	<b>Conclusion</b>
1 (test)	39.0	39.1	39.1	38.9	38.8	39.1	0.1	Non-pyrogenic
2 (test)	39.3	39.3	39.1	38.8	39.1			
3 (test)	39.0	38.7	38.8	39.1	39.4			

2000

*[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]*

2001

There were no adverse clinical findings or animal deaths; or

2002

The following adverse clinical findings or animal deaths occurred:<sup>130</sup>

<i>Description of adverse clinical findings or animal deaths</i>
--

2003

I confirm that:

2004

The above summary information includes all original and any retest data; and

2005

I have checked that there are no differences between the complete test report and this ASCA Summary Test Report.

2006

2007

2008

2009

2010

Name: [TYPED NAME POSITION]

Date

2011

2012

<sup>130</sup> In this situation, the complete test report should be included with ASCA Summary Test Report.. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

2013 **Appendix J: Test Method-Specific ASCA Specifications**  
2014 **and Summary Test Report: Direct and Indirect Hemolysis**  
2015 **(ISO 10993-4 and ASTM F756)**

2016

2017 **A. ASCA Specifications: Direct and Indirect Hemolysis**  
2018 **(ISO 10993-4 and ASTM F756)**

2019

2020 **ISO/IEC 17025 Subclause 6.2(e)**

2021 The procedures, documentation and training program will address the following, at a  
2022 minimum:

- 2023 i. Timing from blood collection to use in test,  
2024 ii. Anticoagulant and concentration used for blood anticoagulation,  
2025 iii. Hemoglobin absorbance standard curve preparation and reporting,  
2026 iv. Dilution procedures and dilution factor calculations,  
2027 v. Sample and control preparation and documentation,  
2028 vi. Correction for test article background interference due to a change in  
2029 color, turbidity, or the presence of particulates,  
2030 vii. Representative sample selection (may apply to both direct and indirect  
2031 contact tests),  
2032 viii. Appropriate cutting and placement of test article samples in test tubes to  
2033 ensure that the entire surface area of the samples is in contact with the  
2034 blood solution (for direct contact test),  
2035 ix. Gentle inversion of tubes approximately every 30 minutes to disperse  
2036 settled red blood cells,  
2037 x. Documentation of supernatant color, turbidity, and presence of particles, if  
2038 any,  
2039 xi. Documentation of presence or absence of red blood cell pellet, pellet size,  
2040 and color after centrifugation if different than negative control,  
2041 xii. Supernatant removal to preserve pellet,  
2042 xiii. Blank sample correction procedures and calculations (including if an  
2043 extract or supernatant has abnormal color or turbidity),  
2044 xiv. Hemolytic index calculation,  
2045 xv. Evaluation criteria and basis for retest,  
2046 xvi. Data documentation, calculations, analysis and result interpretation,  
2047 xvii. Mock study to assess technician competence in test performance, data  
2048 documentation, and result interpretation. A mock study protocol should be  
2049 provided to include, at a minimum, the following:  
2050  
2051 - test and control articles used,  
2052 - test procedure (including recognition and correction of background  
2053 interference in extracts or supernatants),



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- 2054 - how raw and corrected data, documentation of the procedure, analysis,  
2055 and interpretation of results will be captured by the trainee and  
2056 reviewed by the trainer,  
2057 - predefined criteria for assessing a trainee’s performance in the mock  
2058 study to allow them to begin independent ASCA testing.  
2059 xviii. Criteria for technician retraining.  
2060

2061 **ISO/IEC 17025 Subclause 7.7(a)**

2062 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
2063 values will be as follows:

- 2064 i. the positive control material mean hemolytic index is  $\geq 5\%$ ,  
2065 ii. the negative control material mean hemolytic index is  $< 2\%$ ,  
2066 iii. the negative control material “Blank Corrected % Hemolysis” value for any  
2067 replicate is  $\geq -1\%$ .  
2068

2069 **B. Example ASCA Summary Test Report: Direct and**  
2070 **Indirect Hemolysis (ISO 10993-4 and ASTM F756)**

2071  
2072 *Note: This example is intended to illustrate the supplemental documentation that would*  
2073 *accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing*  
2074 *laboratory to the device manufacturer.*  
2075

2076 **Administrative Information**

- 2077 1. Testing Laboratory Name:  
2078 2. ASCA Testing Laboratory Identification Number:  
2079 3. Testing Location(s):  
2080 4. Testing Date(s):  
2081 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
2082  Standard (and particular test method) was \*NOT\* in testing laboratory’s scope of  
2083 *ASCA Accreditation*<sup>131</sup>  
2084  Standard (and particular test method) was in testing laboratory’s scope of *ASCA*  
2085 *Accreditation*  
2086  *ASCA Accreditation* was not suspended  
2087  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

2088  
2089 **ASCA Test Article Prep SOP#: [ASCATAPrep(date/version)]**

<sup>131</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

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- 2090  Test Article was prepared per the above protocol (no deviations/amendments); or  
2091  Test Article was prepared per the above protocol, with the following  
2092 deviations/amendments<sup>132</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

2093 **Test Article:**

- 2094  Entire final finished device  
2095  Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]  
2096 \_\_\_\_\_  
2097 \_\_\_\_\_  
2098  Other:<sup>133</sup> [DESCRIBE]

2099 **Extract testing**

2100 **Extraction Solvent:**

- 2101  Magnesium and Calcium Free PBS  
2102  Other:<sup>134</sup> [DESCRIBE]

2103 **Extraction Ratio:**

- 2104  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*  
2105  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0mm)\*  
2106  1.25 cm<sup>2</sup>/ml (elastomers > 1.0mm thick)\*  
2107  0.2 g/ml (for powder devices)  
2108  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
2109 test article as compared to surface/volume ratio) [*Provide information on comparison*  
2110 *when mass/volume ratio versus surface area/volume ratio is used.*]

2111 \*Note: For absorbent device only: [Specify surface area of test article and the total  
2112 volume of extraction vehicle used taking into account the additional volume from  
2113 absorbency determination.]

- 2114  Other:<sup>135</sup> [DESCRIBE]

2115 **Extraction Conditions:**

- 2116  37°C, 72 h  
2117  50°C, 72 h  
2118  70°C, 24 h  
2119  121°C, 1 h

<sup>132</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>133</sup> In this situation, the complete test report should be included with ASCA Summary Test Report.. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory

<sup>134</sup> *Ibid*

<sup>135</sup> *Ibid*

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2120  Other:<sup>136</sup> [DESCRIBE]

2121

2122 **Agitation During Extraction:**

2123  Extraction with continuous agitation or circulation

2124  Extraction under static conditions or intermittent agitation<sup>137</sup>: [DESCRIBE and PROVIDE  
2125 JUSTIFICATION]

2126

2127 **Fluid Path Extractions:**

2128  For fluid path devices or components (where fluids contact the channels in the device or  
2129 component, and then the fluid enters the body), the extraction was conducted using protocols  
2130 specific to fluid path, with the following approach:<sup>138</sup>

2131  Complete fill with agitation

2132  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

2133  Partial fill with agitation (other surface/volume ratio): [DESCRIBE RATIO USED]

2134  Other: [SUMMARIZE APPROACH]

2135

2136 **Extract Observations (Post-extraction):**

2137  The test article and extract DID NOT change color, and the extract DID NOT appear  
2138 turbid or have particles.

2139  There were changes in color/turbidity or particles in the test article and/or extract OR  
2140 there was swelling/degradation of the test article.<sup>139</sup>

2141

2142 **Post-incubation and centrifugation of blood solutions:**

2143  The test article red blood cell pellet after centrifugation WAS NOT visually different in  
2144 color or size compared to the pellet for the negative control.

2145  The test article red blood cell pellet after centrifugation WAS visually different in color or  
2146 size (e.g., larger or very small) compared to the pellet for the negative control.<sup>140</sup>

2147  The test article supernatant DID NOT change color, appear turbid, or have particles.

2148  There were changes in color/turbidity or particles in the test article supernatant.<sup>141</sup>

---

<sup>136</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>137</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>138</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

<sup>139</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>140</sup> *Ibid*

<sup>141</sup> *Ibid*

*Contains Nonbinding Recommendations*

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2149 **Direct Contact**

2150 **Diluent:**

2151  Magnesium and Calcium Free PBS

2152  Other:<sup>142</sup> [DESCRIBE]

2153 **Exposure Ratio:**

2154  6 cm<sup>2</sup>/ml (<0.5 mm thick)

2155  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)

2156  0.2 g/ml (for powder devices)

2157  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more  
2158 test article as compared to surface/volume ratio) [*Provide information on comparison*  
2159 *when mass/volume ratio versus surface area/volume ratio is used.*]

2160  Other:<sup>143</sup> [DESCRIBE]

2161

2162 **Post-incubation and centrifugation of blood solutions:**

2163  The test article red blood cell pellet after centrifugation WAS NOT visually different in  
2164 color or size compared to the pellet for the negative control.

2165  The test article red blood cell pellet after centrifugation WAS visually different in color or  
2166 size (e.g., larger or very small) compared to the pellet for the negative control.<sup>144</sup>

2167  The test article and supernatant DID NOT change color, and the supernatant DID NOT  
2168 appear turbid or have particles.

2169  There were changes in color/turbidity or particles in the supernatant OR there was  
2170 swelling/degradation of the test article.<sup>145</sup>

2171 **ASCA Test Method SOP #: [ASCAHemolysis(date/version)]**

2172  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
2173 or

2174  Test was conducted per the above protocol and 21 CFR 58, with the following  
2175 deviations/amendments:<sup>146</sup>

<i>Description of deviations/amendments</i>
---

<sup>142</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>143</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>144</sup> *Ibid*

<sup>145</sup> *Ibid*

<sup>146</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

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2176 **Results:**<sup>147</sup>

DRAFT

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<sup>147</sup> The complete test report should be included with ASCA Summary Test Report if one of the following occurs: (1) the negative and positive controls did not perform as expected, (2) the negative control, test article, and blank had absorbance values of 0.000 for all replicates, (3) any replicate of the negative control, test article, or blank samples had a “Blank Corrected % Hemolysis” value less than -1% (e.g., -1.5%), or (4) the final Hemolytic Index of the test article  $\geq 2\%$  .

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

2177

**Table 1 Hemolysis test results<sup>148</sup>**

<b>Extract Hemolysis</b>									
<b>Sample</b>	<b>Absorbance</b>			<b>Blank corrected % hemolysis</b>			<b>Mean Blank Corrected Hemolysis (%)</b>	<b>Hemolytic Index (%)</b>	<b>Conclusions</b>
	<b>Replicate #1</b>	<b>Replicate #2</b>	<b>Replicate #3</b>	<b>Replicate #1</b>	<b>Replicate #2</b>	<b>Replicate #3</b>			
Blank	0.0022	0.0019	0.0026	-0.01	-0.08	0.09	0.00	-	Performed as expected
Negative Control: [Specify]	0.0020	0.0018	0.0019	-0.06	-0.11	-0.08	-0.08	-	Performed as expected
Positive Control: [Specify]	0.3233	0.3258	0.3261	79.68	80.30	80.37	80.11	80.20	Performed as expected
Test	0.0019	0.0015	0.0015	-0.08	-0.18	-0.18	-0.15	-0.07	Non-hemolytic
<b>Direct Hemolysis</b>									
<b>Sample</b>	<b>Absorbance</b>			<b>Blank corrected % hemolysis</b>			<b>Mean Blank Corrected Hemolysis (%)</b>	<b>Hemolytic Index (%)</b>	<b>Conclusions</b>
	<b>Replicate #1</b>	<b>Replicate #2</b>	<b>Replicate #3</b>	<b>Replicate #1</b>	<b>Replicate #2</b>	<b>Replicate #3</b>			

<sup>148</sup> This is an example of how data from a hemolysis test could be presented.

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Blank	0.0057	0.0059	0.0051	0.03	0.08	-0.12	0.00	-	Performed as expected
Negative Control: [Specify]	0.0074	0.0084	0.0103	0.46	0.71	1.18	0.78	-	Performed as expected
Positive Control: [Specify]	0.3732	0.3736	0.3752	91.99	92.09	92.49	92.19	91.41	Performed as expected
Test	0.0096	0.0091	0.0089	1.01	0.88	0.83	0.91	0.13	Non-hemolytic

*[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]*

2178

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2179  
2180  
2181  
2182  
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2192  
2193  
2194

Calibration Coefficient (F): \_\_\_\_\_  
Diluted Blood Hemoglobin Concentration (mg/ml)<sup>149</sup>: \_\_\_\_\_  
Undiluted/Pooled Blood Plasma Free hemoglobin (PFH) (mg/ml): \_\_\_\_\_

I confirm that:

- The above summary information includes all original and any retest data; and
- I have checked that there are no differences between the complete test report and this ASCA Summary Test Report.

---

Name: [TYPED NAME POSITION] Date

---

<sup>149</sup>The complete test report should be included with ASCA Summary Test Report if the total hemoglobin concentration of the diluted blood is outside of the range of 9-11 mg/ml.



2195 **Appendix K: Test Method-Specific ASCA Specifications and**  
2196 **Summary Test Report: SC5b-9 Complement Activation (ISO**  
2197 **10993-4)**

2198

2199 **A. ASCA Specifications: SC5b-9 Complement Activation**  
2200 **(ISO 10993-4)**

2201

2202 **ISO/IEC 17025 Subclause 6.2(e)**

2203 The procedures, documentation and training program will address the following, at a minimum:

- 2204 i. Serum/blood/plasma handling including collection, storage, freeze/thaw (if  
2205 applicable) to minimize complement activation,  
2206 ii. Sample and control preparation and documentation,  
2207 iii. Representative sample selection (from components having direct blood  
2208 contact only),  
2209 iv. Small volume pipetting,  
2210 v. Complement absorbance standard curve,  
2211 vi. Dilution procedures and dilution factor calculations,  
2212 vii. Exposure time,  
2213 viii. Complement concentration calculations,  
2214 ix. Test validation criteria,  
2215 x. Data analysis (including statistical comparison of test sample to positive and  
2216 negative controls) and use of historical control data, if necessary  
2217 xi. Evaluation criteria and basis for retest,  
2218 xii. Data documentation, calculations, analysis and result interpretation,  
2219 xiii. Mock study to assess technician competence in test performance, and data  
2220 documentation, and result interpretation. A mock study protocol should be  
2221 provided to include the following:

2222

- 2223 - test and control articles used,  
2224 - test procedure,  
2225 - how raw data, analysis and result interpretation will be captured by the  
2226 trainee and reviewed by the trainer,  
2227 - predefined criteria for assessing a trainee's performance in the mock study  
2228 to allow them to begin independent ASCA testing.

2229

- 2230 xiv. Criteria for technician retraining.

2231

2232 **ISO/IEC 17025 Subclause 7.7(a)**

2233 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
2234 values will be as follows:

- 2235 i. the positive control meets one of the following criteria:

2236

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- 2237                   ○ the mean value for the cobra venom factor positive control (if applicable)  
2238                   is at least 10X greater than both the mean values for the negative control  
2239                   material and the activated normal human serum, plasma, or whole blood,  
2240                   or  
2241                   ○ the positive material control (if applicable) is statistically significantly  
2242                   higher than both the negative control material and the activated normal  
2243                   human serum, plasma, or whole blood.  
2244  
2245                   ii. any kit-specific high and low controls meet the kit specifications,  
2246                   iii. SC5b-9 complement activation assay validation study protocol and report<sup>150</sup>  
2247                   provided to demonstrate that the test method can clearly differentiate materials  
2248                   with different complement activation potentials (i.e., low, moderate, and high).  
2249

2250                   **B. Example ASCA Summary Test Report: SC5b-9**  
2251                   **Complement Activation (ISO 10993-4)**

2252  
2253                   *Note: This example is intended to illustrate the supplemental documentation that would*  
2254                   *accompany the ASCA DOC. The ASCA Summary Test Report is provided by the testing*  
2255                   *laboratory to the device manufacturer.*  
2256

2257                   **Administrative Information**

- 2258                   1. Testing Laboratory Name:  
2259                   2. ASCA Testing Laboratory Identification Number:  
2260                   3. Testing Location(s):  
2261                   4. Testing Date(s):  
2262                   5. *ASCA Accreditation* Status on the Date(s) of Testing:  
2263                    Standard (and particular test method) was \*NOT\* in testing laboratory’s scope of  
2264                   *ASCA Accreditation*<sup>151</sup>  
2265                    Standard (and particular test method) was in testing laboratory’s scope of *ASCA*  
2266                   *Accreditation*  
2267                    *ASCA Accreditation* was not suspended  
2268                    *ASCA Accreditation* was suspended

2269                   

*Description of reasons for suspension and their impact on testing results.*

<sup>150</sup> As part of the ASCA accreditation application, validation information is needed unless the testing laboratory has a long history of using the test method in regulatory submissions to FDA and that the ELISA kit has been tested on materials with different complement activation potentials.

<sup>151</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

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**Draft – Not for Implementation**

2270 **ASCA Test Article Prep SOP#:** [ASCATAPrep(date/version)]

2271  Test Article was prepared per the above protocol (no deviations/amendments); or

2272  Test Article was prepared per the above protocol, with the following

2273 deviations/amendments<sup>152</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

2274 **Test Article:**

2275  Entire final finished device

2276  Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]

2277

2278  Other: <sup>153</sup>[DESCRIBE]

2279

2280 **US Marketed Comparator Device<sup>154</sup> (optional):**

2281 US marketed comparator device: [DESCRIBE, including the device name, device manufacturer,  
2282 and FDA clearance/approval number]

2283  Entire final finished device

2284  Representative sample selection per SOP. Included/Excluded components: [DESCRIBE]

2285  Other: <sup>155</sup>[DESCRIBE]

2286

2287 **Test Medium:**

2288  Normal Human Serum

2289  Human Plasma

2290  Whole Blood

2291  Other: <sup>156</sup>[DESCRIBE]

2292 **Exposure Ratio:**

2293  6 cm<sup>2</sup>/ml (<0.5 mm thick)

2294  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm)

2295  0.2 g/ml (for powder devices)

<sup>152</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>153</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>154</sup> US-marketed comparator device is a US-marketed device that has a safe use history pertaining to complement activation and has a comparable or larger blood-contacting surface when compared to the subject device. Preferably, a comparator device should have similar intended use and materials of construction as the subject device.

<sup>155</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>156</sup> *Ibid*

***Contains Nonbinding Recommendations***

***Draft – Not for Implementation***

2296  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test  
2297 article as compared to surface/volume ratio) *[Provide information on comparison when*  
2298 *mass/volume ratio versus surface area/volume ratio is used.]*

2299  Other:<sup>157</sup> *[DESCRIBE]*

2300 **Exposure Conditions:**

2301  37°C for 60-90 min

2302  Other:<sup>158</sup> *[DESCRIBE]*

2303  The test article and supernatant DID NOT change color, and the supernatant DID NOT  
2304 appear turbid or have particles.

2305  There were changes in color/turbidity or particles in the supernatant OR there was  
2306 swelling/degradation of the test article.<sup>159</sup>

2307

2308 **ASCA Test Method SOP #:** *[ASCAComplement(date/version)]*

2309  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or

2310  Test was conducted per the above protocol and 21 CFR 58, with the following  
2311 deviations/amendments:<sup>160</sup>

*Description of deviations/amendments*

2312 Manufacturer of SC5b-9 ELISA kit: \_\_\_\_\_

2313 **Results:**<sup>161</sup>

---

<sup>157</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>158</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>159</sup> *Ibid*

<sup>160</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Device Manufacturer may also be requested to provide a rationale to support a regulatory decision.

<sup>161</sup> The complete test report should be included with ASCA Summary Test Report if test medium, negative, positive, and comparator controls did not perform as expected, or there was a statistically significant increase in SC5b-9 for test article compared to negative or comparator controls.

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2314

**Table 1 SC5b-9 Protein Concentration (ng/mL)<sup>162</sup>**

Samples	Dilution	Concentration (ng/mL)					Conclusion
		Replicate #1	Replicate #2	Replicate #3	Mean	Std	
Test Article	1:160	208	216	212	212	3.8	Not a complement activator*
Test Medium Control [ <i>Specify (e.g., blood, serum, or plasma)</i> ]	1:160	205	207	208	207	1.5	Performed as expected
Negative Control Material: [ <i>Specify</i> ]	1:160	206	205	204	205	1.1	Performed as expected
Positive Control Material: <sup>163</sup> [ <i>Specify</i> ]	1:160	683	693	688	688	4.8	Performed as expected
Cobra Venom Factor Positive Control <sup>164</sup>	1:8000	10326	10567	10519	10471	127	Performed as expected
US marketed comparator (optional)	1:160	210	215	223	216	6.6	Performed as expected

2315

*[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]*

2316

\*not statistically different from negative or comparator controls

2317

2318 I confirm that:

2319  The above summary information includes all original and any retest data; and

2320  I have checked that there are no differences between the complete test report and this ASCA  
2321 Summary Test Report.

2322

2323

2324

2325 Name: [TYPED NAME POSITION]

Date

2326

<sup>162</sup> This is an example of how data from a complement activation test could be presented.

<sup>163</sup> Depending on the positive control used, this row may be relevant.

<sup>164</sup> *Ibid*

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**2327 Appendix L: Example ASCA Test Specific Controls**

2328 *This example is intended to illustrate test-specific controls (positive, negative, reagent) that*  
 2329 *could impact the validity of ASCA tests for which we recommend purchase control and/or*  
 2330 *verification testing specifications be established.*

2331  
 2332

<b>ASCA Test Method</b>	<b>Control/Reagent</b>
MEM Elution Cytotoxicity	Positive Control (e.g., Latex)
	Negative Control (e.g., HDPE)
	Elution Medium (e.g., MEM)
	Animal Serum (e.g., Fetal Bovine Serum (FBS))
	[Other Reagents or Controls]
Hemolysis	Positive Control (e.g., Latex)
	Negative Control (e.g., HDPE)
	Ca/Mg-free PBS
	Hemoglobin Reagent
	Drabkin's Reagent
	Hemoglobin Standard Solution
	[Other Reagents or Controls]
Guinea Pig Maximization Sensitization (GPMT)	Positive Control (e.g., DNCB)
	Solvent in which the positive control is dissolved
	0.9% Sodium Chloride
	Sesame Oil (if used as vehicle control)
	Cottonseed Oil (if used as vehicle control)
	Sodium Dodecyl Sulfate/Sodium Lauryl Sulfate (SLS)
	Vehicle in which SLS is reconstituted (e.g., USP petroleum jelly)
	Freund's Complete Adjuvant (FCA)
[Other Reagents or Controls]	
Intracutaneous Reactivity	0.9% Sodium Chloride
	Positive Control (e.g., SLS)
	Sesame Oil (if used as vehicle control)
	Cottonseed Oil (if used as vehicle control)
	[Other Reagents or Controls]
Acute Systemic Toxicity	0.9% Sodium Chloride
	Sesame Oil (if used as vehicle control)
	Cottonseed Oil (if used as vehicle control)
	[Other Reagents or Controls]
Material Mediated Pyrogenicity	0.9% Sterile "Pyrogen-Free" Saline (e.g., 0.9% USP Sodium Chloride for Injection)
Dermal Irritation	0.9% Sodium Chloride
	Positive Control (e.g., SLS)
	Negative Control (e.g., gauze)
	Solvent in which the positive control is dissolved
	Sesame Oil (if used as vehicle control for extract testing)
	Cottonseed Oil (if used as vehicle control for extract testing)
Closed Patch Sensitization	Positive Control (e.g., DNCB)
	Negative Control (e.g., gauze)
	Solvent in which the positive control is dissolved
	0.9% Sodium Chloride
	Sesame Oil (if used as vehicle control for extract testing)

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	Cottonseed Oil (if used as vehicle control for extract testing)
	[Other Reagents or Controls]
SC5b-9 Complement Activation	SC5b-9 Kit (e.g., model and supplier)
	Positive Control (e.g., Cobra Venom Factor)
	Positive Control Material (e.g., Latex)
	Negative Control Material (e.g., LDPE)
	Test Medium (e.g., Normal Human Serum, Human Plasma)
	[Other Reagents or Controls]

2333

2334

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2335 **Appendix M: Test Method-Specific ASCA Specifications and**  
2336 **Summary Test Report –MTT Cytotoxicity (ISO 10993-5)**

2337 **A. ASCA Specifications: MTT Cytotoxicity (ISO 10993-5)**

2338 **ISO/IEC 17025 Subclause 6.2(e)**

2339 The procedures, documentation, and training program will address the following, at a minimum:

- 2340 i. Cell line<sup>165</sup> maintenance (e.g., cell line subculture, cell line storage, storage conditions,  
2341 cell line recovery from storage, use of mycoplasma-free cell line, good cell culture  
2342 practices, morphology assessment),  
2343 ii. Cell counting,  
2344 iii. Cell seeding,  
2345 iv. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) solution preparation  
2346 and storage,  
2347 v. Check for cell seeding errors and growth characteristics of control and treated cells,  
2348 vi. Multichannel pipetting,  
2349 vii. Cell culture medium removal from the 96 well plates,  
2350 viii. Addition of test and control samples to the cell cultures,  
2351 ix. Preparation of different doses of test extract and positive controls, if needed,  
2352 x. Microscopic evaluation of cell morphology,  
2353 xi. MTT solution addition and removal,  
2354 xii. Use of microplate reader and calibration,  
2355 xiii. Evaluation criteria and basis for retest,  
2356 xiv. Data documentation, calculations, analysis and result interpretation,  
2357 xv. Mock study to assess technician competence in test performance, data documentation,  
2358 and results interpretation. A mock study protocol should be provided to include, at a  
2359 minimum, the following:  
2360 - test and control articles used,  
2361 - test and control article preparation if this task is conducted by the trainee,  
2362 - how test samples and controls are blinded to the trainee,  
2363 - test procedure,  
2364 - how raw data, analysis and result interpretation will be captured by the trainee and  
2365 reviewed by the trainer, and  
2366 - predefined criteria for assessing a trainee's performance in the mock study to allow  
2367 them to begin independent ASCA testing.  
2368 xvi. Criteria for technician retraining.

2369  
2370 **ISO/IEC 17025 Subclause 7.7(a)**

2371 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
2372 values will be as follows<sup>166</sup>:

---

<sup>165</sup>L929 cell line is recommended for ASCA testing. Other cell lines may be considered if, as part of the ASCA accreditation application, the testing laboratory provides documentation and a justification (i.e., based on a validation report, historical use for FDA submissions) to FDA to support the use of another cell line for MTT cytotoxicity testing.

<sup>166</sup> See ISO 10993-5 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.



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- 2373 iv. The mean OD<sub>570</sub> of media control (i.e., media with cells) is  $\geq 0.2$ ,  
2374 v. The means of the “Left Media Control” and “Right Media Control” do not differ by more  
2375 than 15% of the mean of all media control replicates,  
2376 vi. The viability of each positive control material (e.g., ZDEC or ZDBC<sup>167</sup>) replicate is less  
2377 than 70% of the mean of all media control replicates,  
2378 vii. The viability of each negative control replicate is greater than 70% of the mean of all  
2379 media control replicates, and  
2380 viii. The viability of 50% extract dose of each positive control material (e.g., ZDEC or  
2381 ZDBC) replicate has at least the same or higher viability than the 100% extract dose of  
2382 the positive control.  
2383

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<sup>167</sup> ZDEC and ZDBC are organotin-stabilized polyurethanes.

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2384 **B. Example ASCA Summary Test Report: MTT Cytotoxicity**  
2385 **(ISO 10993-5)**

2386 *Note: This example is intended to illustrate the supplemental documentation that would*  
2387 *accompany the ASCA DOC. The ASCA summary test report is provided by the testing laboratory*  
2388 *to the device manufacturer.*

2389 **Administrative Information**

- 2390 1. Testing Laboratory Name:  
2391 2. ASCA Testing Laboratory Identification Number:  
2392 3. Testing Location(s):  
2393 4. Testing Date(s):  
2394 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
2395  Standard (and particular test method) was **NOT** in testing laboratory's scope of *ASCA*  
2396 *Accreditation*<sup>168</sup>  
2397  Standard (and particular test method) was in testing laboratory's scope of *ASCA*  
2398 *Accreditation*  
2399  *ASCA Accreditation* was not suspended  
2400  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

2401

2402 **ASCA Test Article Prep SOP#:** [ASCATAPrep(date/version)]

2403  Test Article was prepared per the above protocol (no deviations/amendments); or

2404  Test Article was prepared per the above protocol, with the following

2405 deviations/amendments<sup>169</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

2406 **Test Article:**

2407  Entire final finished device

2408  Representative sample selection per SOP. Included/Excluded components: [Describe]

2409  Other:<sup>170</sup> [Describe]

2410

<sup>168</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of *ASCA Accreditation*.

<sup>169</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>170</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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#### 2411 **Extraction Solvent:**

2412  MEM with 5-10% animal serum [*Specify concentration and source (e.g., 5% fetal bovine*

2413 *serum)*]

2414  Other:<sup>171</sup> [*Describe*]

2415

#### 2416 **Extraction Ratio:**

2417  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

2418  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)\*

2419  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

2420  0.2 g/ml (for powder devices)

2421  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test

2422 article as compared to surface/volume ratio)

2423 \*For absorbent device: [*Specify surface area of test article and the total volume of extraction*

2424 *vehicle used taking into account the additional volume from absorbency determination*]

2425  Other:<sup>172</sup> [*Describe*]

2426

#### 2427 **Extraction Conditions:**

2428  37°C, 24 h

2429  37°C, 72 h

2430  Other:<sup>173</sup> [*Describe*]

2431

#### 2432 **Agitation During Extraction:**

2433  Extraction with continuous agitation or circulation

2434  Extraction under static conditions or intermittent agitation<sup>174</sup>: [*Describe and provide*

2435 *justification*]

2436

#### 2437 **Fluid Path Extractions:**

2438  For fluid path devices or components (where fluids contact the channels in the device or

2439 component, and then the fluid enters the body), the extraction was conducted using protocols

2440 specific to fluid path, with the following approach:<sup>175</sup>

2441  Complete fill with agitation

2442  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

2443  Partial fill with agitation (other surface/volume ratio): [*Describe ratio used*]

2444  Other: [*Summarize approach*]

2445

2446

---

<sup>171</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>172</sup> *Ibid*

<sup>173</sup> *Ibid*

<sup>174</sup> *Ibid*

<sup>175</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

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2447 **Test Article and Extract Appearance:**

2448  The test article and extract DID NOT change color, and the extract DID NOT appear turbid or  
2449 have particles.

2450  There were changes in color/turbidity or particles in the test article and/or extract OR there  
2451 was swelling/degradation of the test article.<sup>176</sup>

2452

*Describe changes in the test article and/or extract and, if applicable, provide justification to support why changes in the test article and/extract are acceptable.*

2453 **ASCA Test Method SOP #:** *[ASCACytotoxMTT(date/version)]* \_\_\_\_\_

2454  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or

2455  Test was conducted per the above protocol and 21 CFR 58, with the following  
2456 deviations/amendments:<sup>177</sup>

*Description of deviations/amendments*

2457

2458

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<sup>176</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>177</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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2459 **Results:**

**Table 1: Optical Density (OD) and Cell Viability Data <sup>178</sup>**

2460  
2461  
2462

	Optical Density (*OD <sub>570</sub> )					†Viability (%)
	+Rep.1	Rep.2	Rep.3	Mean	^SD(±)	
Test Article Extract (100%)						
Test Article Extract (e.g., 75%)						
Test Article Extract (e.g., 50%)						
Test Article Extract (e.g., 25%)						
Positive Control Extract (e.g., ZDBC 100% extract)						
Positive Control Extract (e.g., 75%)						
Positive Control Extract (e.g., 50%)						
Positive Control Extract (e.g., 25%)						
Negative Control Extract (e.g., ‡HDPE 100% extract)						

2463

	Optical Density (OD <sub>570</sub> )				
	Rep. 1	Rep. 2	Rep. 3	Mean	Difference from the Total Mean of All Media Controls
Left Media Control					
Right Media Control					
Total Mean of All Media Controls					N/A

2464 \*OD<sub>570</sub>: optical density reading with a 570 nm filter with a reference wavelength of 650 nm.

2465 +Rep.: Replicate, at least three replicates should be used for each control and test article extract.  
2466 If more replicates are used, add more columns.

2467 ^SD: Standard Deviation

2468 †Viability% =  $\frac{\text{mean value of } OD_{570} \text{ of the test article extract}}{\text{mean value of } OD_{570} \text{ of the medium control}} \times 100\%$

2469 ‡ HDPE: high-density polyethylene

2470

<sup>178</sup> Per ISO 10993-5:2009 “Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity,” Annex C, optical density and cell viability are measured after 24 hr. incubation of test and control articles with cells. If tests are conducted for a 48 hr. or 72 hr. incubation, validation report should be provided to support the validity of the assay.

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2471

**Table 2: Microscopic Evaluation of Cells (optional)**

	Scoring Grades (e.g., per Table 1, ISO 10993-5:2009)		
	*Rep. 1	Rep. 2	Rep.3
Test Article Extract (100%)			
Test Article Extract (e.g., 75%)			
Test Article Extract (e.g., 50%)			
Test Article Extract (e.g., 25%)			
Positive Control Extract (e.g., ZDBC 100% extract)			
Positive Control Extract (e.g., 75%)			
Positive Control Extract (e.g., 50%)			
Positive Control Extract (e.g., 25%)			
Negative Control Extract (e.g., HDPE 100% extract)			

2472

2473

2474

2475

\*Rep.: Replicate, at least three replicates should be used for each control and test article extract.  
If more replicates are used, add more columns.

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2476 **I confirm that the following test validity criteria<sup>179</sup> are met:**  
2477

- 2478  The mean OD<sub>570</sub> of the media control (i.e., media with cells) is  $\geq 0.2$ .  
2479  The means of the “Left Media Control” and “Right Media Control” do not differ by more  
2480 than 15% of the mean of all media control replicates.  
2481  The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than  
2482 70% of the mean of all media control replicates.  
2483  The viability of each negative control replicate is greater than 70% of the mean of all media  
2484 control replicates.  
2485  The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC)  
2486 replicate has at least the same or higher viability than the 100% extract dose of the positive  
2487 control.  
2488  The viability of the 50% extract dose of each test article replicate has at least the same or  
2489 higher viability than the 100% extract dose of the test article.

2490  
2491 **Overall Results:**  
2492

- 2493  Cytotoxic<sup>180</sup>. Any individual replicate of the test article extract (100%) or dilution of the test  
2494 article extract resulted in a % viability lower than 70%.  
2495  Non-cytotoxic. All individual replicate of the test article extract (100%) or dilution of the test  
2496 article extract resulted in a % viability 70% or greater.  
2497

2498 I confirm that:

- 2499  
2500  The above summary information includes all original and any retest data; and  
2501  I have checked that there are no differences between the complete test report and this ASCA  
2502 summary test report.

2503 \_\_\_\_\_  
2504 Name: [TYPED NAME POSITION]

\_\_\_\_\_ Date

<sup>179</sup> If any of the test validity criteria are not met, retesting should be conducted and the complete test report should be included with the ASCA Summary Test Report.

<sup>180</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

2505 **Appendix N: Test Method-Specific ASCA Specifications and**  
2506 **Summary Test Report –Neutral Red Uptake (NRU)**  
2507 **Cytotoxicity (ISO 10993-5)**

2508 **A. ASCA Specifications NRU Cytotoxicity (ISO 10993-5)**

2509 **ISO/IEC 17025 Subclause 6.2(e)**

2510 The procedures, documentation and training program will address the following, at a minimum:

- 2511 i. Cell line<sup>181</sup> maintenance (e.g., cell line subculture, cell line storage, storage conditions,  
2512 cell line recovery from storage, use of mycoplasma-free cell line, good cell culture  
2513 practices, morphology assessment),  
2514 ii. Quality check for growth-stimulating properties of newborn calf serum (NBCS) lots,  
2515 iii. Cell counting,  
2516 iv. Cell seeding,  
2517 v. Preparation and storage of: Neutral red (NR) stock solution, NR medium, and  
2518 ethanol/acetic acid solution (NRdesorb),  
2519 vi. Check for cell seeding errors and growth characteristics of control and treated cells,  
2520 vii. Multichannel pipetting,  
2521 viii. Cell culture medium removal from the 96 well plates,  
2522 ix. Addition of test and control samples to the cell cultures,  
2523 x. Preparation of different doses of test extract and positive controls, if needed.  
2524 xi. Cell washing,  
2525 xii. Addition and removal of NR medium,  
2526 xiii. PBS solution removal,  
2527 xiv. NRdesorb incubation,  
2528 xv. Use of microplate reader and calibration,  
2529 xvi. Evaluation criteria and basis for retest,  
2530 xvii. Data documentation, calculations, IC<sub>50</sub> value (inhibitory concentration estimated to affect  
2531 the endpoint in question by 50%) determination, analysis and result interpretation,  
2532 xviii. Mock study to assess technician competence in test performance, data documentation,  
2533 and result interpretation. A mock study protocol should be provided to include, at a  
2534 minimum, the following:  
2535 - test and control articles used,  
2536 - test and control article preparation if this task is conducted by the trainee,  
2537 - how test samples and controls are blinded to the trainee,  
2538 - test procedure,  
2539 - how raw data, analysis and result interpretation will be captured by the trainee and  
2540 reviewed by the trainer, and  
2541 - predefined criteria for assessing a trainee's performance in the mock study to allow  
2542 them to begin independent ASCA testing.

---

<sup>181</sup>BALB/c 3T3 cell line is recommended for ASCA testing. Other cell lines may be considered if, as part of the ASCA accreditation application, the testing laboratory provides documentation and a justification (i.e., based on a validation report, historical use for FDA submissions) to FDA to support the use of another cell line for NRU cytotoxicity testing.



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2543 xix. Criteria for technician retraining.

2544

2545 **ISO/IEC 17025 Subclause 7.7(a)**

2546 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
2547 values will be as follows<sup>182</sup>:

- 2548 i. The mean OD<sub>540</sub> of the media controls (i.e., media with cells) is  $\geq 0.3$ ,
- 2549 ii. The means of the “Left Media Control” and “Right Media Control” do not differ by more  
2550 than 15% of the mean of all media control replicates,
- 2551 iii. The IC<sub>50</sub> for Sodium Lauryl Sulfate (SLS) falls within the 95% confidence interval of  
2552 0.093 mg/mL (i.e., between 0.07 mg/mL to 0.116 mg/mL),
- 2553 iv. The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less  
2554 than 70% of the mean of all media control replicates,
- 2555 v. The viability of each negative control replicate is greater than 70% of the mean of all  
2556 media control replicates, and
- 2557 vi. The viability of the 50% extract dose of each positive control material (e.g., ZDEC or  
2558 ZDBC) replicate has at least the same or higher viability than the 100% extract dose of  
2559 the positive control.
- 2560

---

<sup>182</sup> See ISO 10993-5 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.

2561 **B. Example ASCA Summary Test Report: Neutral Red**  
2562 **Uptake (NRU) Cytotoxicity (ISO 10993-5)**

2563 *Note: This example is intended to illustrate the supplemental documentation that would*  
2564 *accompany the ASCA DOC. The ASCA summary test report is provided by the testing laboratory*  
2565 *to the device manufacturer.*

2566 **Administrative Information**

- 2567 1. Testing Laboratory Name:  
2568 2. ASCA Testing Laboratory Identification Number:  
2569 3. Testing Location(s):  
2570 4. Testing Date(s):  
2571 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
2572  Standard (and particular test method) was **NOT** in testing laboratory’s scope of *ASCA*  
2573 *Accreditation*<sup>183</sup>  
2574  Standard (and particular test method) was in testing laboratory’s scope of *ASCA*  
2575 *Accreditation*  
2576  *ASCA Accreditation* was not suspended  
2577  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

2578  
2579 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

- 2580  Test Article was prepared per the above protocol (no deviations/amendments); or  
2581  Test Article was prepared per the above protocol, with the following  
2582 deviations/amendments<sup>184</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

2583 **Test Article:**

- 2584  Entire final finished device  
2585  Representative sample selection per SOP. Included/Excluded components: *[Describe]*  
2586  Other:<sup>185</sup> *[Describe]*  
2587

<sup>183</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

<sup>184</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>185</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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#### 2588 **Extraction Solvent:**

2589  Dulbecco's Modification of Eagle's Medium (DMEM) with 5% newborn calf serum  
2590 (NBCS)

2591  Other:<sup>186</sup> [Describe]

2592

#### 2593 **Extraction Ratio:**

2594  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

2595  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)\*

2596  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

2597  0.2 g/ml (for powder devices)

2598  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test  
2599 article as compared to surface/volume ratio)

2600 \*For absorbent device: [Specify surface area of test article and the total volume of extraction  
2601 vehicle used taking into account the additional volume from absorbency determination]

2602  Other:<sup>187</sup> [Describe]

2603

#### 2604 **Extraction Conditions:**

2605  37°C, 24 h

2606  37°C, 72 h

2607  Other:<sup>188</sup> [Describe]

2608

#### 2609 **Agitation During Extraction:**

2610  Extraction with continuous agitation or circulation

2611  Extraction under static conditions or intermittent agitation<sup>189</sup>: [Describe and provide  
2612 justification]

2613

#### 2614 **Fluid Path Extractions:**

2615  For fluid path devices or components (where fluids contact the channels in the device or  
2616 component, and then the fluid enters the body), the extraction was conducted using protocols  
2617 specific to fluid path, with the following approach:<sup>190</sup>

2618  Complete fill with agitation

2619  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

2620  Partial fill with agitation (other surface/volume ratio): [Describe ratio used]

2621  Other: [Summarize approach]

2622

2623

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<sup>186</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>187</sup> *Ibid*

<sup>188</sup> *Ibid*

<sup>189</sup> *Ibid*

<sup>190</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

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***Draft – Not for Implementation***

2624 **Test Article and Extract Appearance:**

2625  The test article and extract DID NOT change color, and the extract DID NOT appear turbid or  
2626 have particles.

2627  There were changes in color/turbidity or particles in the test article and/or extract OR there  
2628 was swelling/degradation of the test article.<sup>191</sup>

2629

*Describe changes in the test article and/or extract and, if applicable, provide justification to support why changes in the test article and/extract are acceptable.*

2630

2631 **ASCA Test Method SOP #:** [*ASCACytotoxNRU(date/version)*]

2632  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58;  
2633 or

2634  Test was conducted per the above protocol and 21 CFR 58, with the following  
2635 deviations/amendments.<sup>192</sup>

2636

*Description of deviations/amendments*

2637

2638

2639

---

<sup>191</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>192</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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2640 **Results:**

2641

2642

2643

**Table 1: Optical Density and Cell Viability Data<sup>193</sup>**

	Optical Density (*OD <sub>540</sub> )					†Viability (%)	IC50
	+Rep.1	Rep.2	Rep.3	Mean	^SD(±)		
Test Article Extract (100%)							N/A
Test Article Extract (e.g., 75%)							
Test Article Extract (e.g., 50%)							
Test Article Extract (e.g., 25%)							
Positive Control Extract (e.g., ZDBC 100% extract)							N/A
Positive Control Extract (e.g., 75%)							
Positive Control Extract (e.g., 50%)							
Positive Control Extract (e.g., 25%)							
SLS (0.05 mg/mL)							
SLS (0.1 mg/mL)							
SLS (0.15 mg/mL)							
SLS (0.2 mg/mL)							
Negative Control Extract (e.g., ‡HDPE 100% extract)							N/A

2644

	Optical Density (OD <sub>540</sub> )					Difference from the Total Mean of All Media Controls
	Rep.1	Rep.2	Rep.3	Mean		
Left Media Control						
Right Media Control						
Total Mean of All Media Controls						

2645 \*OD<sub>540</sub>: optical density reading with a 540 nm filter using the blanks as a reference.

2646 +Rep.: Replicate, at least three replicates should be used for each control and test article extract.

2647 If more replicates are used, add more columns.

2648 ^SD: Standard Deviation

2649 †Viability% =  $\frac{\text{mean value of } OD_{540} \text{ of the test article extract}}{\text{mean value of } OD_{540} \text{ of the medium control}} \times 100\%$

2650 ‡ HDPE: high-density polyethylene

2651

<sup>193</sup> Per ISO 10993-5:2009 “Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity,” Annex A, optical density and cell viability are measured after 24 hr. incubation of test and control articles with cells. If tests are conducted for a 48 hr. or 72 hr. incubation, validation report should be provided to support the validity of the assay.

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2652  
2653

**Table 2: Microscopic Evaluation of Cells (optional)**

	<i>Scoring Grades (e.g., per Table 1, ISO 10993-5:2009)</i>		
	<b>*Rep.1</b>	<b>Rep.2</b>	<b>Rep.3</b>
Test Article Extract (100%)			
Test Article Extract (e.g., 75%)			
Test Article Extract (e.g., 50%)			
Test Article Extract (e.g., 25%)			
Positive Control Extract (e.g., ZDBC 100% extract)			
Positive Control Extract (e.g., 75%)			
Positive Control Extract (e.g., 50%)			
Positive Control Extract (e.g., 25%)			
Sodium Lauryl Sulfate (0.05 mg/mL)			
Sodium Lauryl Sulfate (0.1 mg/mL)			
Sodium Lauryl Sulfate (0.15 mg/mL)			
Sodium Lauryl Sulfate (0.2 mg/mL)			
Negative Control Extract (e.g., HDPE 100% extract)			

2654  
2655  
2656  
2657

\*Rep.: Replicate, at least three replicates should be used for each control and test article extract. If more replicates are used, add more columns.

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2658 **I confirm that the following test validity criteria<sup>194</sup> are met:**

- 2659
- 2660  The mean OD<sub>540</sub> of the media control (i.e., media with cells) is  $\geq 0.3$ .
- 2661  The means of the “Left Media Control” and “Right Media Control” do not differ by more
- 2662 than 15% of the mean of all media control replicates.
- 2663  The IC 50 for SLS falls within the 95% confidence interval of 0.093 mg/ml (i.e., between
- 2664 0.07 mg/mL to 0.116 mg/mL).
- 2665  The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than
- 2666 70% of the mean of all media control replicates.
- 2667  The viability of each negative control replicate is greater than 70% of the mean of all media
- 2668 control replicates.
- 2669  The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC)
- 2670 replicate has at least the same or higher viability than the 100% extract dose of the positive
- 2671 control.
- 2672  The viability of the 50% extract dose of each test article replicate has at least the same or
- 2673 higher viability than the 100% extract dose of the test article.
- 2674

2675 **Overall Results:**

- 2676
- 2677  Cytotoxic<sup>195</sup>. Any individual replicate of the test article extract (100%) or dilution of the test
- 2678 article extract resulted in a % viability lower than 70%.
- 2679  Non-cytotoxic. All individual replicate of the test article extract (100%) or dilution of the test
- 2680 article extract resulted in a % viability 70% or greater.

2681 I confirm that:

- 2682
- 2683  The above summary information includes all original and any retest data; and
- 2684  I have checked that there are no differences between the complete test report and this ASCA
- 2685 summary test report.

2686 Name: [TYPED NAME POSITION]

Date

2687  
2688

---

<sup>194</sup> If any of the test validity criteria are not met, retesting should be conducted and the complete test report should be included with the ASCA Summary Test Report.

<sup>195</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

2689 **Appendix O: Test Method-Specific ASCA Specifications and**  
2690 **Summary Test Report –XTT Cytotoxicity (ISO 10993-5)**

2691 **A. ASCA Specifications: XTT Cytotoxicity (ISO 10993-5)**

2692 **ISO/IEC 17025 Subclause 6.2(e)**

2693 The procedures, documentation and training program will address the following, at a minimum:

- 2694 i. Cell line<sup>196</sup> maintenance (e.g., cell line subculture, cell line storage, storage conditions,  
2695 cell line recovery from storage, use of mycoplasma-free cell line, good cell culture  
2696 practices, morphology assessment),  
2697 ii. Cell counting,  
2698 iii. Cell seeding,  
2699 iv. Preparation and storage of XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-  
2700 tetrazolium-5-carboxyaniline inner salt) solution and PMS (phenazine methosulfate)  
2701 solution,  
2702 v. Check for cell seeding errors and growth characteristics of control and treated cells,  
2703 vi. Multichannel pipetting,  
2704 vii. Cell culture medium removal from the 96 well plates,  
2705 viii. Addition of test and control samples to the cell cultures,  
2706 ix. Preparation of different doses of test extract and positive controls, if needed.  
2707 x. Microscopic evaluation of cell morphology,  
2708 xi. Addition of XTT/PMS solution mixture to cells and incubation,  
2709 xii. Transfer of test, control, and blank samples for absorbance reading,  
2710 xiii. Use of microplate reader, calibration, and blank correction,  
2711 xiv. Evaluation criteria and basis for retest,  
2712 xv. Data documentation, calculations, analysis and result interpretation,  
2713 xvi. Mock study to assess technician competence in test performance, data documentation,  
2714 and result interpretation. A mock study protocol should be provided to include, at a  
2715 minimum, the following:  
2716 - test and control articles used,  
2717 - test and control article preparation if this task is conducted by the trainee,  
2718 - how test samples and controls are blinded to the trainee,  
2719 - test procedure,  
2720 - how raw data, analysis and result interpretation will be captured by the trainee and  
2721 reviewed by the trainer, and  
2722 - predefined criteria for assessing a trainee's performance in the mock study to allow  
2723 them to begin independent ASCA testing.  
2724 xvii. Criteria for technician retraining.  
2725

---

<sup>196</sup>L929 cell line is recommended for ASCA testing. Other cell lines may be considered if, as part of the ASCA accreditation application, the testing laboratory provides documentation and a justification (i.e., based on a validation report, historical use for FDA submissions) to FDA to support the use of another cell line for MTT cytotoxicity testing.



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2726 **ISO/IEC 17025 Subclause 7.7(a)**

2727 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
2728 values will be as follows<sup>197</sup>:

- 2729 i. The mean OD<sub>450</sub> of the media control (i.e., media with cells) is  $\geq 0.2$ ,  
2730 ii. The means of the “Left Media Control” and “Right Media Control” do not differ by more  
2731 than 15% of the mean of all media control replicates,  
2732 iii. The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less  
2733 than 70% of the mean of all media control replicates,  
2734 iv. The viability of each negative control replicate is greater than 70% of the mean of all  
2735 media control replicates, and  
2736 v. The viability of the 50% extract dose of each positive control material (e.g., ZDEC or  
2737 ZDBC) replicate has at least the same or higher viability than the 100% extract dose of  
2738 the positive control.  
2739

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<sup>197</sup> See ISO 10993-5 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

2740 **B. Example ASCA Summary Test Report: XTT Cytotoxicity**  
2741 **(ISO 10993-5)**

2742 *Note: This example is intended to illustrate the supplemental documentation that would*  
2743 *accompany the ASCA DOC. The ASCA summary test report is provided by the testing laboratory*  
2744 *to the device manufacturer.*

2745 **Administrative Information**

- 2746 1. Testing Laboratory Name:  
2747 2. ASCA Testing Laboratory Identification Number:  
2748 3. Testing Location(s):  
2749 4. Testing Date(s):  
2750 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
2751  Standard (and particular test method) was **NOT** in testing laboratory's scope of *ASCA*  
2752 *Accreditation*<sup>198</sup>  
2753  Standard (and particular test method) was in testing laboratory's scope of *ASCA*  
2754 *Accreditation*  
2755  *ASCA Accreditation* was not suspended  
2756  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

2757  
2758 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

- 2759  Test Article was prepared per the above protocol (no deviations/amendments); or  
2760  Test Article was prepared per the above protocol, with the following  
2761 deviations/amendments<sup>199</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

2762 **Test Article:**

- 2763  Entire final finished device  
2764  Representative sample selection per SOP. Included/Excluded components: *[Describe]*  
2765  Other:<sup>200</sup> *[Describe]*

2766 **Extraction Solvent:**

- 2767  MEM with 5-10% animal serum *[Specify the concentration and source (e.g., 5% fetal*  
2768 *bovine serum)]*

<sup>198</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of *ASCA Accreditation*.

<sup>199</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>200</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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2769  Other:<sup>201</sup> [Describe]

2770 **Extraction Ratio:**

2771  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

2772  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)\*

2773  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

2774  0.2 g/ml (for powder devices)

2775  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test article as compared to surface/volume ratio)

2777 \*For absorbent device: [Specify surface area of test article and the total volume of extraction vehicle used taking into account the additional volume from absorbency determination]

2778  Other:<sup>202</sup> [Describe]

2780

2781 **Extraction Conditions:**

2782  37°C, 24 h

2783  37°C, 72 h

2784  Other:<sup>203</sup> [Describe]

2785

2786 **Agitation During Extraction:**

2787  Extraction with continuous agitation or circulation

2788  Extraction under static conditions or intermittent agitation<sup>204</sup>: [Describe and provide justification]

2789

2791 **Fluid Path Extractions:**

2792  For fluid path devices or components (where fluids contact the channels in the device or component, and then the fluid enters the body), the extraction was conducted using protocols specific to fluid path, with the following approach:<sup>205</sup>

2795  Complete fill with agitation

2796  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

2797  Partial fill with agitation (other surface/volume ratio): [Describe ratio used]

2798  Other: [Summarize approach]

2799

2800

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<sup>201</sup> *Ibid*

<sup>202</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>203</sup> *Ibid*

<sup>204</sup> *Ibid*

<sup>205</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

2801 **Test Article and Extract Appearance:**

2802  The test article and extract DID NOT change color, and the extract DID NOT appear turbid or  
2803 have particles.

2804  There were changes in color/turbidity or particles in the test article and/or extract OR there  
2805 was swelling/degradation of the test article.<sup>206</sup>

2806

*to support why changes in the test article and/extract are acceptable.*

2807

2808 **ASCA Test Method SOP #:** [ASCACytotoxXTT(date/version)]

2809  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or

2810  Test was conducted per the above protocol and 21 CFR 58, with the following

2811 deviations/amendments:<sup>207</sup>

*Description of deviations/amendments*

2812

2813

2814

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<sup>206</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>207</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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2815 **Results:**

2816

**Table 1: Optical Density and Cell Viability Data<sup>208</sup>**

2817

	<b>*Blank Corrected Optical Density (OD<sub>450</sub>)</b>					<b>†Viability (%)</b>
	<b>+Rep. 1</b>	<b>Rep. 2</b>	<b>Rep. 3</b>	<b>Mean</b>	<b>^SD. (±)</b>	
Test Article Extract (100%)						
Test Article Extract (e.g., 75%)						
Test Article Extract (e.g., 50%)						
Test Article Extract (e.g., 25%)						
Positive Control Extract (e.g., ZDBC 100% extract)						
Positive Control Extract (e.g., 75%)						
Positive Control Extract (e.g., 50%)						
Positive Control Extract (e.g., 25%)						
Negative Control Extract (e.g., ‡HDPE 100% extract)						

2818

	<b>Optical Density (OD<sub>450</sub>)</b>				
	<b>Rep. 1</b>	<b>Rep. 2</b>	<b>Rep. 3</b>	<b>Mean</b>	<b>Difference from the Total Mean of All Media Controls</b>
Left Media Control					
Right Media Control					
Total Mean of All Media Controls					N/A

2819

2820 \*Blank corrected optical density (OD<sub>450</sub>): Optical density reading with a 450 nm filter and a  
2821 reference wavelength of 630 nm using cell culture media without cells for blank correction.

2822 Blank is cell culture media without cells.

2823 +Rep.: Replicate, at least three replicates should be used for each control and test article extract.

2824 If more replicates are used, add more columns.

2825 ^SD: Standard Deviation

2826 †Viability% =  $\frac{\text{mean value of } OD_{450} \text{ of the test article extract}}{\text{mean value of } OD_{450} \text{ of the medium control}} \times 100\%$

2827 ‡ HDPE: high-density polyethylene

2828

<sup>208</sup> Per ISO 10993-5:2009 “Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity,” Annex D, optical density and cell viability are measured after 24 hr. incubation of test and control articles with cells. If tests are conducted for a 48 hr. or 72 hr. incubation, validation report should be provided to support the validity of the assay.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

2829  
2830

**Table 2: Microscopic Evaluation of Cells (optional)**

	Microscopic Evaluation of Cells		
	*Rep. 1	Rep. 2	Rep.3
Test Article Extract (100%)			
Test Article Extract (e.g., 75%)			
Test Article Extract (e.g., 50%)			
Test Article Extract (e.g., 25%)			
Positive Control Extract (e.g., ZDBC 100% extract)			
Positive Control Extract (e.g., 75%)			
Positive Control Extract (e.g., 50%)			
Positive Control Extract (e.g., 25%)			
Negative Control Extract (e.g., HDPE 100% extract)			

2831  
2832  
2833  
2834

\*Rep.: Replicate, at least three replicates should be used for each control and test article extract. If more replicates are used, add more columns.

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2835 **I confirm that that the following test validity criteria<sup>209</sup> are met:**  
2836

- 2837  The mean OD<sub>450</sub> of the media control (i.e., media with cells) is  $\geq 0.2$ .  
2838  The means of the “Left Media Control” and “Right Media Control” do not differ by more  
2839 than 15% of the mean of all media control replicates.  
2840  The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than  
2841 70% of the mean of all media control replicate.  
2842  The viability of each negative control replicate is greater than 70% of the mean of all media  
2843 control replicates.  
2844  The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC)  
2845 replicate has at least the same or higher viability than the 100% extract dose of the positive  
2846 control.  
2847  The viability of the 50% extract dose of each test article replicate has at least the same or  
2848 higher viability than the 100% extract dose of the test article.  
2849

2850 **Overall Results:**  
2851

- 2852  Cytotoxic<sup>210</sup>. Any individual replicate of the test article extract (100%) or dilution of the test  
2853 article extract resulted in a % viability lower than 70%.  
2854  Non-cytotoxic. All individual replicates of the test article extract (100%) or dilution of the test  
2855 article extract resulted in a % viability of 70% or greater.

2856 I confirm that:

- 2857  
2858  The above summary information includes all original and any retest data; and  
2859  I have checked that there are no differences between the complete test report and this ASCA  
2860 summary test report.

2861 Name: [TYPED NAME POSITION]  
2862

Date  
2863

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<sup>209</sup> If any of the test validity criteria are not met, retesting should be conducted and the complete test report should be included with ASCA Summary Test Report.

<sup>210</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

2864 **Appendix P: Test Method-Specific ASCA Specifications and**  
2865 **Summary Test Report –Bacterial Reverse Mutation Test**  
2866 **(ISO 10993-3 and OECD 471<sup>211</sup>)**

2867 **A. ASCA Specifications: Bacterial Reverse Mutation Assay**  
2868 **(i.e., Ames Assay) (ISO 10993-3 and OECD 471)**

2869 **ISO/IEC 17025 Subclause 6.2(e)**

2870 The procedures, documentation and training program will address the following, at a minimum:

- 2871 i. Bacterial cell culture techniques including stock culture preparation, and bacterial culture  
2872 maintenance and storage,  
2873 ii. Bacterial strain phenotypic marker verification,  
2874 iii. Verification of cell density before testing,  
2875 iv. Verification of spontaneous revertant colony plate counts,  
2876 v. Verification of plate sterility,  
2877 vi. Preparation of medium, test article and test article extracts, and controls (i.e., negative  
2878 and positive),  
2879 vii. Preparation and quality control of S9 fraction,  
2880 viii. Plate preparation,  
2881 ix. Exposure of bacterial cells to controls and test article extracts (e.g., plate incorporation  
2882 method, preincubation method),  
2883 x. Criteria for use of preincubation method vs. plate incorporation method,  
2884 xi. Use and calibration of automated colony counters, if used,  
2885 xii. Revertant colony counting,  
2886 xiii. Evaluation of bacterial background lawn,  
2887 xiv. Interpretation of results,  
2888 xv. Mock study to assess technician competence in test performance, data documentation,  
2889 and result interpretation. A mock study protocol should be provided to include, at a  
2890 minimum, the following:  
2891 - test article, negative (vehicle) controls, and positive controls used,  
2892 - preparation of test article, negative controls, and positive controls, if this task is  
2893 conducted by the trainee,  
2894 - how test samples and controls are blinded to the trainee,  
2895 - test procedure,  
2896 - revertant colony counting, colony sizing, and discrimination of small colonies vs.  
2897 large colonies,  
2898 - how raw data, analysis and result interpretation will be captured by the trainee and  
2899 reviewed by the trainer, and

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<sup>211</sup> OECD stands for Organisation for Economic Co-operation and Development. See <http://www.oecd.org> for more information. OECD 471 “Guidelines for Testing of Chemicals – Bacterial Reverse Mutation Test.” All OECD guidelines (OECD 471, OECD 490) referenced in this document are incorporated by reference in ISO 10993-3 “Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity.”



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- 2900 - predefined criteria for assessing a technician's performance in the mock study to  
2901 allow them to begin independent ASCA testing.
- 2902 xvi. Criteria for technician retraining.
- 2903
- 2904 **ISO/IEC 17025 Clause 7.2 c)**
- 2905 7.2 c) The testing laboratory agrees that test procedures will include or specify, as appropriate,  
2906 the following:
- 2907 i. Test system (i.e., bacterial strains) used, which should include at least 5 bacterial strains  
2908 with the following combination of strains:
- 2909 a. Salmonella typhimurium TA1535, and  
2910 b. Salmonella typhimurium TA1537 or TA97 or TA97a, and  
2911 c. Salmonella typhimurium TA98, and  
2912 d. Salmonella typhimurium TA100, and  
2913 e. Escherichia coli WP2 uvrA, or Escherichia coli WP2 uvrA (pKM101), or  
2914 Salmonella typhimurium TA102.
- 2915 ii. Cell density (e.g., approximately  $10^9$  cells/ml) and confirmation of cell density prior to  
2916 testing,
- 2917 iii. Procedure for phenotypic characterization of tester strains prior to testing,
- 2918 iv. Procedure for use of plate incorporation method or/and preincubation method,
- 2919 v. Procedure for establishing spontaneous revertant rate for each tester strain,
- 2920 vi. Test medium, including a description of medium preparation,
- 2921 vii. S9 fraction (a cofactor-supplemented post-mitochondrial fraction) type (e.g., rat liver  
2922 S9 fraction), source of S9 fraction (e.g., in-house prepared or name of commercial  
2923 supplier and product name), and how S9 fraction is qualified (e.g., Certificate of  
2924 Analysis of the product used, verification testing) before testing. If prepared in-house,  
2925 specify the preparation method, rodent strain, enzyme-inducing agents (e.g., Aroclor  
2926 1254), concentration of cofactors, purity, and verification criteria for S9 activity. For  
2927 the S9 mixture (e.g., S9 fraction and cofactors), specify the amount or percentage of S9  
2928 fraction and concentration of co-factors in the mixture, and the source of the S9 mixture  
2929 (e.g., in-house or name of commercial supplier and product name).
- 2930 viii. Procedure to ensure absence of microbial contamination prior to plating,
- 2931 ix. Procedure for test article preparation and extract storage,
- 2932 x. Procedure and criteria for cytotoxicity determination of test extract<sup>212</sup>. The test extract  
2933 is considered cytotoxic if one or both of the following criteria are met for all tester  
2934 strains:
- 2935 • Greater than 50% reduction in the number of revertant colonies,  
2936 • At least a moderate reduction in the background lawn.
- 2937 xi. Procedure for exposure of bacterial cells to the test article and controls with and  
2938 without S9,
- 2939 xvii. Procedure for use of undiluted extracts unless cytotoxicity is observed, in which case,  
2940 dilutions of extracts should also be tested,

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<sup>212</sup> If cytotoxicity is observed with the test extract, a complete test report should be provided.

## *Contains Nonbinding Recommendations*

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- 2941 xii. Procedure for colony counting,  
2942 xiii. Procedure for preparation of positive and negative (vehicle) concurrent controls  
2943 including final concentrations (e.g., amount of positive control per plate). Negative  
2944 controls should be handled in a manner similar to the device extract (i.e., incubated  
2945 under the same conditions).  
2946 xiv. Procedure for generation of historical negative (vehicle) and positive control data (e.g.,  
2947 ranges, means and standard deviations), criteria for inclusion and exclusion of historical  
2948 studies in the historical data cohorts, and how the expected range of revertant colony  
2949 plate counts is established based on historical negative (vehicle) and positive control  
2950 data<sup>213</sup>. The historical control data set should initially be built with at least 20  
2951 experiments conducted under comparable testing conditions as used for medical device  
2952 testing<sup>214</sup>. The distribution of the data together with appropriate descriptive statistics  
2953 should be provided (e.g., confidence intervals, 95-99% percentiles).  
2954 xv. Procedure for when repeat test is needed.  
2955

#### **ISO/IEC 17025 Subclause 7.7(a)**

2956 The testing laboratory agrees that pre-defined criteria for positive/negative/reference control  
2957 values will be as follows:  
2958

- 2959 i. The phenotypic characterization of each tester strain demonstrates appropriate genetic  
2960 markers per OECD 471,  
2961 ii. Cell density for each tester strain before testing should be approximately  $1 \times 10^9$  cells ml,  
2962 iii. Each concurrent negative (vehicle) control has spontaneous revertant colony plate counts  
2963 for each tester strain within the frequency ranges of the laboratory's historical control  
2964 data and within the range reported in the literature,  
2965 iv. The mean of the number of revertant colonies in each positive control should exhibit  
2966 significant increase compared to that in the respective solvent control,  
2967 v. Each concurrent positive control material replicate is at least 3 times that of the  
2968 concurrent negative control for each tester strain<sup>215</sup>,  
2969 vi. The background lawns of negative (vehicle) controls are normal per ISO/TR 10993-33:  
2970 2015<sup>216</sup>, Table 2,  
2971 vii. Test extract is considered acceptable based on the cytotoxicity evaluation criteria as  
2972 defined in Section 7.2 c). x. above.  
2973

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<sup>213</sup>Testing laboratories should have historical data available when applying for ASCA accreditation, and this data should be consistent with the literature.

<sup>214</sup> Hayashi, M, et.al., Compilation and Use of Genetic Toxicity Historical Control Data, Mutation Res., 2011, 723 (2): 87-90.

<sup>215</sup> If other criteria for positive controls are used when applying for ASCA accreditation, a justification should be provided based on testing laboratory's historical data.

<sup>216</sup> ISO/TR 10993-33: 2015 Biological evaluation of medical devices Part 33: Guidance on tests to evaluate genotoxicity Supplement to ISO 10993-3.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

2974 **B. Example ASCA Summary Test Report: Bacterial Reverse**  
2975 **Mutation Assay (i.e., Ames Assay) (ISO 10993-3 and OECD**  
2976 **471)**

2977 *Note: This example is intended to illustrate the supplemental documentation that would*  
2978 *accompany the Declaration of Conformity per FDA’s guidance [Appropriate Use of Voluntary](#)*  
2979 *[Consensus Standards in Premarket Submissions for Medical Devices](#). The ASCA summary test*  
2980 *report is provided by the testing laboratory to the device manufacturer.*

2981 **Administrative Information**

- 2982 1. Testing Laboratory Name:  
2983 2. ASCA Testing Laboratory Identification Number:  
2984 3. Testing Location(s):  
2985 4. Testing Date(s):  
2986 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
2987  Standard (and particular test method) was **NOT** in testing laboratory’s scope of *ASCA*  
2988 *Accreditation*<sup>217</sup>  
2989  Standard (and particular test method) was in testing laboratory’s scope of *ASCA*  
2990 *Accreditation*  
2991  *ASCA Accreditation* was not suspended  
2992  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

2993  
2994 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

2995  Test Article was prepared per the above protocol (no deviations/amendments); or

2996  Test Article was prepared per the above protocol, with the following  
2997 deviations/amendments<sup>218</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

2998 **Test Article:**

2999  Entire final finished device

3000  Representative sample selection per SOP. Included/Excluded components: *[Describe]*

3001  Other:<sup>219</sup> *[Describe]*

3002

<sup>217</sup> See FDA’s guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory’s scope of *ASCA Accreditation*.

<sup>218</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>219</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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#### 3003 Extraction Solvent:

- 3004  0.9% Saline
- 3005  Dimethyl Sulfoxide (DMSO)
- 3006  Ethanol [*Provide justification for using ethanol instead of DMSO (e.g., documentation of*
- 3007 *device degradation in DMSO)*]
- 3008  Polyethylene glycol 400 (PEG 400) [*Provide justification for using PEG instead of DMSO*
- 3009 *(e.g., documentation of device degradation in DMSO)*]
- 3010  Other:<sup>220</sup> [*Describe*]

#### 3011

#### 3012 Extraction Ratio:

- 3013  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*
- 3014  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)\*
- 3015  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*
- 3016  0.2 g/ml (for powder devices)
- 3017  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test
- 3018 article as compared to surface/volume ratio)
- 3019 \*For absorbent device: [*Specify surface area of test article and the total volume of extraction*
- 3020 *vehicle used taking into account the additional volume from absorbency determination*]
- 3021  Other:<sup>221</sup> [*Describe*]

#### 3022

#### 3023 Extraction Conditions:

- 3024  37°C, 72 h
- 3025  50°C, 72 h
- 3026  70°C, 24 h
- 3027  121°C, 1 h
- 3028  Other:<sup>222</sup> [*Describe*]

#### 3029

#### 3030 Agitation During Extraction:

- 3031  Extraction with continuous agitation or circulation
- 3032  Extraction under static conditions or intermittent agitation<sup>223</sup>: [*Describe and provide*
- 3033 *justification*]

3034

3035

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<sup>220</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>221</sup> *Ibid*

<sup>222</sup> *Ibid*

<sup>223</sup> *Ibid*

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3036 **Fluid Path Extractions:**

3037  For fluid path devices or components (where fluids contact the channels in the device or  
3038 component, and then the fluid enters the body), the extraction was conducted using protocols  
3039 specific to fluid path, with the following approach<sup>224</sup>:

3040  Complete fill with agitation

3041  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

3042  Partial fill with agitation (other surface/volume ratio): *[Describe ratio used]*

3043  Other: *[Summarize approach]*

3044

3045 **Test Article and Extract Appearance:**

3046  The test article and extract DID NOT change color, and the extract DID NOT appear turbid or  
3047 have particles.

3048  There were changes in color/turbidity or particles in the test article and/or extract OR there  
3049 was swelling/degradation of the test article.<sup>225</sup>

3050

*Describe changes in the test article and/or extract and, if applicable, provide justification to support why changes in the test article and/or extract are acceptable.*

3051

3052 **ASCA Test Method SOP #:** *[#####-ASCAAmes(date/version)]*

3053  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or

3054  Test was conducted per the above protocol and 21 CFR 58, with the following  
3055 deviations/amendments:<sup>226</sup>

3056

*Description of deviations/amendments*

3057

3058

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<sup>224</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

<sup>225</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>226</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

*Contains Nonbinding Recommendations*

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3059 Results  
3060  
3061

**Table 1: Tester Strain Phenotypic Characterization\***

Bacterial Species	Strains	Ampicillin resistance	Tetracycline Resistance	Sensitivity to UV radiation	Sensitivity to crystal violet	Histidine requirement for growth	Tryptophan requirement for growth
Salmonella Typhimurium	<input type="checkbox"/> TA98						
	<input type="checkbox"/> TA1535						
	<input type="checkbox"/> TA100						
	<input type="checkbox"/> TA97						
	<input type="checkbox"/> TA97a						
	<input type="checkbox"/> TA1537						
	<input type="checkbox"/> TA102						
Escherichia coli	<input type="checkbox"/> WP2 uvrA						
	<input type="checkbox"/> WP2 uvrA (pKM101)						

3062 (\*Select strains that are used in testing and record phenotypic characterization results for each bacterial strain. If the phenotypic test is  
3063 not applicable to the strain, the box is greyed out)  
3064  
3065

3066 **I confirm that the bacterial counts for each tester strain before testing were:**

3067  
3068  Approximately  $1 \times 10^9$  cells/ml<sup>227</sup>  
3069

<sup>227</sup> See OECD 471 Guidelines for Testing of Chemicals – Bacterial Reverse Mutation Test. If bacterial counts for any tester strain before testing do not meet the criteria, retesting should be conducted.

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3070 **Table 2: Positive Control [specify positive control] and concentration for each tester strain]**  
3071

Tester Strain	Positive Control (with S9)	Concentration (e.g., in µg per plate)
Tester Strain	Positive Control (without S9)	Concentration (e.g., in µg per plate)

3072 **Table 3: Historical Positive and Negative Control Colony Count Data [specify testing**  
3073 **period] \***  
3074  
3075

Tester Strain	Negative Control Colony Counts			Positive Control Colony Counts		
	Range	Mean ±SD	Number of Data Points	Range	Mean ±SD	Number of Data Points
<b>Without S9</b>						
<b>With S9</b>						

3076  
3077 \* Historical negative and positive control data should be built with at least 20 experiments (i.e.,  
3078 20 data points for each tester strain with and without S9 fraction) under comparable testing  
3079 conditions as used for medical device testing.

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3080

**Table 4: Test Article and Concurrent Control Colony Count Data**

	□Replicate Number	With S9 Activation					Without S9 Activation				
		*Test Article Polar Extract	*Polar Vehicle Control	*Test Article Non-Polar Extract	*Non-polar Vehicle Control	Positive Control	*Test Article Polar Extract	*Polar Vehicle Control	*Test Article Non-Polar Extract	*Non-Polar Vehicle Control	Positive Control
Strain 1 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
	Mean										
	·SD										
	†FI										
Strain 2 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
	Mean										
	·SD										
	†FI										
Strain 3 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
	Mean										
	·SD										
	†FI										
Strain 4 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
	Mean										
	SD										
	FI										
Strain 5 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
	Mean										
	·SD										
	†FI										



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3081

3082 \*Specify test article polar extract (e.g., test article saline extract), polar vehicle control (e.g., saline), test article non-polar extract (e.g.,  
3083 test article DMSO extract), non-polar vehicle control (e.g., DMSO), and positive control in the results table per SOP. Commonly used  
3084 polar extraction vehicle is 0.9% saline. Commonly used non-polar extraction vehicle is DMSO, ethanol, and polyethylene glycol 400  
3085 (PEG 400)<sup>228</sup>.

3086 □At least 3 replicates should be used for each test article and control.

3087 ·SD=Standard Deviation

3088 †FI=Fold increase =  $\frac{\text{mean test article colony count value}}{\text{mean vehicle control colony count value}}$

3089

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<sup>228</sup> Per ISO 10993-18 “Biological evaluation of medical devices Part 18: Chemical characterization of medical device materials within a risk management process,” ethanol, DMSO, and polyethylene glycol are semi-polar solvents. The term non-polar used in this document follows ISO 10993-3 where genotoxicity tests are discussed.

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**Table 5: Background Lawn<sup>◇</sup> Observation<sup>229</sup>**

3090  
3091

		With S9 Activation					Without S9 Activation				
		*Test Article Polar Extract	*Polar Vehicle Control	*Test Article Non-polar Extract	*Non-polar Vehicle Control	Positive Control	*Test Article Polar Extract	*Polar Vehicle Control	*Test Article Non-polar Extract	*Non-polar Vehicle Control	Positive Control
Strain 1 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
Strain 2 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
Strain 3 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
Strain 4 [Specify]	Rep.1										
	Rep.2										
	Rep.3										
Strain 5 [Specify]	Rep.1										
	Rep.2										
	Rep.3										

3092 \* Specify test article polar extract (e.g., test article saline extract), polar vehicle control (e.g., saline), test article non-polar extract  
3093 (e.g., test article DMSO extract), non-polar vehicle control (e.g., DMSO), and positive control in the results table per SOP.

3094 <sup>◇</sup> Describe background lawn using criteria defined in Table 2 of ISO/TR 10993-33: normal (1), slightly reduced (2), moderately  
3095 reduced (3), extremely reduced (4), etc.

<sup>229</sup> Complete test report should be included and repeat testing may be needed if moderately reduced [or worse] background lawn (i.e., grade 2 or above per Table 2 of ISO/TR 10993-33 background lawn evaluation criteria) is observed in any of the tester strains with either test extract or controls. The testing laboratory/manufacturer should also provide a rationale to support a regulatory decision.

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3096 **I confirm that that the following test validity criteria are met<sup>230</sup>:**  
3097

- 3098  The phenotypic characterization of each tester strain demonstrates normal results.  
3099  Each concurrent negative control has spontaneous revertant colony plate counts for each  
3100 tester strain within the frequency ranges of the laboratory's historical control data and  
3101 within the range reported in the literature.  
3102  The mean of the number of revertant colonies in each positive control shows significant  
3103 increase compared with that of the respective solvent control.  
3104  Each concurrent positive control material replicate is at least 3 times the concurrent  
3105 negative control for each tester strain<sup>231</sup>.  
3106  The background lawns of negative controls are normal.  
3107  The test extract is considered acceptable based on the cytotoxicity evaluation criteria as  
3108 defined in Section 7.2 c. x.) above.  
3109

3110 **Overall Results:**  
3111

- 3112  The test article demonstrated a positive response in one or more tester strains when tested with  
3113 or without S9 fraction<sup>232</sup>. The results are positive if the mean mutant colony counts are two  
3114 or more times greater than the respective concurrent negative control mean for the tester  
3115 strains TA98, TA100, TA 97, TA 102 and the two E. coli strains and three or more times  
3116 greater than the respective concurrent negative control mean for the tester strains TA1535  
3117 and TA1537.  
3118  The test article demonstrated a negative response in all tester strains when tested with or  
3119 without S9 fraction. The results are negative if the mean mutant colony counts are less than  
3120 two times of the respective concurrent negative control mean for the tester strains TA98,  
3121 TA100, TA 97, TA 102 and the two E. coli strains and less than three times of the respective  
3122 concurrent negative control mean for the tester strains TA1535 and TA1537.  
3123  The test article demonstrated an equivocal response (i.e., elevated mutant colony counts that  
3124 don't meet the criteria for a positive response) in one or more tester strains under any test  
3125 conditions (i.e., with or without S9 fraction)<sup>233</sup>.

*Provide justification to support why an equivocal response is acceptable and repeat testing is not needed. Alternatively, provide repeat test data with the tester strains under the test conditions that previously showed an equivocal response, and explain the findings.*

3126  
3127

---

<sup>230</sup> If any of the test validity criteria are not met, retesting should be conducted and the complete test report should be included with ASCA Summary Test Report.

<sup>231</sup> If other criteria for positive controls are used, when applying for ASCA accreditation, a justification should be provided based on testing laboratory's historical data.

<sup>232</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. The testing laboratory or manufacturer should also provide a rationale to support a regulatory decision

<sup>233</sup> *Ibid*

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3128 I confirm that:

3129

3130  The above summary information includes all original and any retest data; and

3131  I have checked that there are no differences between the complete test report and this ASCA  
3132 summary test report.

---

3133 Name: [TYPED NAME POSITION]

Date

3134

DRAFT

3135 **Appendix Q: Test Method-Specific ASCA Specifications and**  
3136 **Summary Test Report –Mouse Lymphoma Assay (MLA)**  
3137 **(ISO 10993-3 and OECD 490<sup>234</sup>)**

3138 **A. ASCA Specifications: MLA (ISO 10993-3 and OECD 490)**

3139 **ISO/IEC 17025 Subclause 6.2(e)**

3140 The procedures, documentation and training program will address the following, at a minimum:

- 3141 i. Mouse lymphoma cell line<sup>235</sup> maintenance including checks for: mycoplasma  
3142 contamination, karyotype stability of the cells, modal number of chromosomes, and  
3143 doubling time; and cleansing of pre-existing mutant cells prior to use in the assay,  
3144 ii. Cell culture media, cloning media, test article, and control (negative and positive)  
3145 preparation,  
3146 iii. Preparation and quality control of S9 fraction,  
3147 iv. Treatment of cells with test and control articles,  
3148 v. Techniques for cell washing and dilution, determination of viable cell concentration, and  
3149 adjustment to study-specified cell concentrations,  
3150 vi. Cell cloning and determination of cloning efficiency,  
3151 vii. Soft agar method (if applicable):  
3152 - Cell and cloning agar media mixing with and without the mutant selective agent (e.g.,  
3153 trifluorothymidine (TFT)),  
3154 - Colony counting (i.e., number of colonies), colony sizing, and criteria for small  
3155 colonies vs. large colonies  
3156 viii. Microwell method (if applicable):  
3157 - Cell plating into 96 well plates with and without the mutant selective agent (e.g.,  
3158 TFT),  
3159 - Colony counting (i.e., number of wells with colonies), and discrimination of small  
3160 colonies vs. large colonies  
3161 ix. Cytotoxicity measurement, including determination of relative total growth,  
3162 x. Determination of relative total growth, relative cloning efficiency, induced mutant  
3163 frequency, small colony mutant frequency,  
3164 xi. Evaluation criteria and basis for retest,  
3165 xii. Data documentation, calculations, analysis and result interpretation,  
3166 xiii. Mock study to assess technician competence in test performance, data documentation,  
3167 and result interpretation. A mock study protocol should be provided to include, at a  
3168 minimum, the following:  
3169 - test and control articles used,  
3170 - test and control article preparation if this task is conducted by the trainee,  
3171 - how test samples and controls are blinded to the trainee,

---

<sup>234</sup> OECD 490 Guidelines for the Testing of Chemicals – In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene.

<sup>235</sup> Only L5178Y TK+/- -3.7.2C mouse lymphoma cell line (generally called L5178Y) is allowed for MLA under ASCA.

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- 3172 - colony counting, colony sizing, and discrimination of small colonies vs. large  
3173 colonies,  
3174 - test procedure,  
3175 - how raw data, analysis and result interpretation will be captured by the trainee and  
3176 reviewed by the trainer,  
3177 - predefined criteria for assessing a technician's performance in the mock study to  
3178 allow them to begin independent ASCA testing.  
3179 xiv. Technician retraining, if needed.

#### 3180 **ISO/IEC 17025 Clause 7.2 c)**

3181 7.2 c) The testing laboratory agrees that test procedures will include or specify, as appropriate,  
3182 the following:

- 3183 i. Cell density prior to dosing and during expression period,  
3184 ii. Procedure for cell culture maintenance and quality control of mouse lymphoma cells  
3185 including identity, absence of mycoplasma, sensitivity to positive control, stable  
3186 spontaneous mutant frequency,  
3187 iii. Stock culture preparation, including periodic cleansing of spontaneous mutant cells,  
3188 iv. Test medium including a description of medium preparation (e.g., serum concentration,  
3189 S9 fraction concentration),  
3190 v. S9 fraction (a cofactor-supplemented post-mitochondrial fraction) type (e.g., rats liver  
3191 S9 fraction), source of S9 fraction (e.g., in-house prepared or name of commercial  
3192 supplier and product name), and how S9 fraction is qualified (e.g., Certificate of  
3193 Analysis of the product used, verification testing) before testing. If prepared in-house,  
3194 specify the preparation method, rodent strain, enzyme-inducing agents (e.g., Aroclor  
3195 1254), concentration of cofactors, purity, and verification criteria for S9 activity. For  
3196 the S9 mixture (e.g., S9 fraction and cofactors), specify the amount or percentage of S9  
3197 fraction and co-factors in the mixture, and the source of the S9 mixture (e.g., in-house  
3198 or name of commercial supplier and product name).  
3199 vi. Procedure for test article extract preparation and extract storage,  
3200 vii. Procedure for test article extract dose preparation. For example, if RPMI medium is  
3201 used for extraction, the undiluted extract supplemented with serum should be used for  
3202 testing. If 0.9% saline is used for extraction, dilution to 10% (v/v) in RPMI medium  
3203 with serum should be used for testing. If non-polar solvents are used for extraction,  
3204 dilution to 1% (v/v) in RPMI medium with serum should be used for testing. For mouse  
3205 lymphoma assay, commonly used non-polar solvents for extraction are DMSO, ethanol,  
3206 PEG 400<sup>236</sup>.  
3207 viii. Procedure for concurrent control (positive, negative) preparations, including final  
3208 concentrations. Negative controls should be handled in a manner similar to the device  
3209 extract (i.e., incubated under the same conditions).

---

<sup>236</sup> Per ISO 10993-18 "Biological evaluation of medical devices Part 18: Chemical characterization of medical device materials within a risk management process," ethanol, DMSO, and polyethylene glycol are semi-polar solvents. The term non-polar used in this document follows ISO 10993-3 where genotoxicity tests are discussed.

## *Contains Nonbinding Recommendations*

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- 3210 ix. Procedure for exposure of cells to test articles and controls (4-hour treatment with and  
3211 without S9, and 24-hour treatment without S9),
- 3212 x. Procedure and criteria for cytotoxicity determination of test extract and positive  
3213 controls (i.e., relative total growth),
- 3214 xi. Procedure for mutant expression and cloning,
- 3215 xii. Procedure for plating cells into soft agar medium (for soft agar method) or into 96 well  
3216 plate (for microwell method),
- 3217 - For soft agar method, approximately 200 cells per plate in soft agar medium  
3218 should be seeded in petri plates (designated as viable cell (VC) plates) and  $1 \times 10^6$   
3219 cells per plate in TFT-containing soft agar medium should be seeded in petri  
3220 plates (designated as TFT plates) for mutant selection<sup>237</sup>. At a minimum, three  
3221 plates per replicate sample (test and control) should be used for colony count for  
3222 both VC plates and TFT plates.
- 3223 - For microwell method, approximately 1.6 cells per well in cloning medium  
3224 should be seeded in 96 well plates (designated as viable cell (VC) plates) and  
3225 approximately 2000 cells per well in TFT-containing cloning medium should be  
3226 seeded in 96 well plates (designated as TFT plates) for mutant selection<sup>238</sup>. At a  
3227 minimum, two 96-well plates per replicate sample (test and control) should be  
3228 used for VC plates colony count and four 96-well plates per replicate sample (test  
3229 and control articles) should be used for TFT plates colony count.
- 3230 xiii. Procedure to determine optimal dose for testing (i.e., dose dependent study), if  
3231 cytotoxicity is observed in any of the extracts,
- 3232 xiv. Procedures for colony counting, colony sizing, and discrimination of small colonies vs.  
3233 large colonies,
- 3234 xv. Procedure for generation of historical negative (i.e., solvent/vehicle control) and  
3235 positive control data (e.g., ranges, means and standard deviations), criteria for inclusion  
3236 and exclusion of historical studies in the historical data cohorts, and how the expected  
3237 range of mutant frequencies is established based on historical negative and positive  
3238 control data<sup>239</sup>. The historical control data set should initially be built with at least 20  
3239 experiments conducted under comparable testing conditions, as used for medical device  
3240 testing. The distribution of the data, together with appropriate descriptive statistics  
3241 should be provided (e.g., confidence intervals, 95-99% percentiles),
- 3242 xvi. Criteria for repeat testing,  
3243  
3244

---

<sup>237</sup> Mei, et.al., Methods for Using the Mouse Lymphoma Assay to Screen for Chemical Mutagenicity and Photo-Mutagenicity, Mutation Res., Methods in Pharmacology and Toxicology, 2014

<sup>238</sup> *Ibid*

<sup>239</sup> Testing laboratories should have historical positive and negative control data available when applying for ASCA accreditation, and this data should be consistent with the literature. See OECD 490 “Guidelines for the Testing of Chemicals – In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene,” on how to establish historical database for negative and positive controls.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

3245 **ISO/IEC 17025 Subclause 7.7(a)**

3246 The testing laboratory agrees that pre-defined criteria for positive and negative control values  
3247 will be as follows:

3248 i. The negative control meets the following criteria:

Parameter	Soft Agar Method	Microwell Method
Mutant Frequency (MF)	35 – 140 X 10 <sup>-6</sup>	50 – 170 X 10 <sup>-6</sup>
Cloning Efficiency (CE)	65 – 120%	65 – 120%
Suspension Growth (SG)	8– 32 fold (3-4 hour treatment) 32 – 180 fold (24-hour treatment)	8 – 32 fold (3-4 hour treatment) 32 – 180 fold (24-hour treatment)

3249  
3250 ii. The positive controls meet at least one of the following two acceptance criteria for both  
3251 4-hr (with and without S9) and 24-hr (without S9) assays:

- 3252 - The positive control demonstrates an absolute increase in total MF, that is, an  
3253 increase above the spontaneous background MF. The increase in MF [i.e., the  
3254 induced MF (IMF)] should be at least 300 X 10<sup>-6</sup>. The small colony IMF should be  
3255 compared to the total IMF, and should be at least 40% of the total IMF.
- 3256 - The positive control has an increase in the small colony MF of at least 150 X 10<sup>-6</sup>  
3257 above that seen in the concurrent negative control (i.e., the small colony IMF of 150  
3258 X 10<sup>-6</sup>).

3259 iii. The relative total growth (RTG) of cells treated with positive controls should be 10% or  
3260 greater.

3261  
3262



*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

3263 **B. Example ASCA Summary Test Report: In Vitro Mouse**  
3264 **Lymphoma Test (ISO 10993-3 and OECD 490) — Soft Agar**  
3265 **Method**

3266 **Administrative Information**

- 3267 1. Testing Laboratory Name:  
3268 2. ASCA Testing Laboratory Identification Number:  
3269 3. Testing Location(s):  
3270 4. Testing Date(s):  
3271 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
3272  Standard (and particular test method) was **NOT** in testing laboratory's scope of *ASCA*  
3273 *Accreditation*<sup>240</sup>  
3274  Standard (and particular test method) was in testing laboratory's scope of *ASCA*  
3275 *Accreditation*  
3276  *ASCA Accreditation* was not suspended  
3277  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

3278  
3279 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

- 3280  Test Article was prepared per the above protocol (no deviations/amendments); or  
3281  Test Article was prepared per the above protocol, with the following  
3282 deviations/amendments<sup>241</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

3283 **Extraction Solvent:**

- 3284  RPMI medium (without serum)  
3285  RPMI medium (with serum) *[Specify serum concentration per SOP and provide*  
3286 *justification (e.g., all other non-polar solvents are not compatible with test article for*  
3287 *extraction)]*  
3288  0.9% Saline *[Provide justification for using 0.9% Saline instead of RPMI medium without*  
3289 *serum]*  
3290  DMSO  
3291  PEG 400 *[Provide justification for using PEG instead of DMSO (e.g., documentation of*  
3292 *device degradation in DMSO)]*

<sup>240</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of *ASCA Accreditation*.

<sup>241</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

## Contains Nonbinding Recommendations

### Draft – Not for Implementation

3293  Ethanol [*Provide justification for using ethanol instead of DMSO (e.g., documentation of*  
3294 *device degradation in DMSO)*]

3295  Other:<sup>242</sup> [*Describe*]

#### 3296 **Extraction Ratio:**

3297  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

3298  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)\*

3299  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

3300  0.2 g/ml (for powder devices)

3301  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test  
3302 article as compared to surface/volume ratio)

3303 \*For absorbent device: [*Specify surface area of test article and the total volume of extraction*  
3304 *vehicle used taking into account the additional volume from absorbency determination)*]

3305  Other:<sup>243</sup> [*Describe*]

3306

#### 3307 **Extraction Conditions:**

3308  37°C, 72 h

3309  50°C, 72 h

3310  70°C, 24 h

3311  121°C, 1 h

3312  Other:<sup>244</sup> [*Describe*]

3313

#### 3314 **Agitation During Extraction:**

3315  Extraction with continuous agitation or circulation

3316  Extraction under static conditions or intermittent agitation<sup>245</sup>: [*Describe and provide*  
3317 *justification*] \_\_\_\_\_

3318

#### 3319 **Fluid Path Extractions:**

3320  For fluid path devices or components (where fluids contact the channels in the device or  
3321 component, and then the fluid enters the body), the extraction was conducted using protocols  
3322 specific to fluid path, with the following approach:<sup>246</sup>

3323  Complete fill with agitation

3324  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

3325  Partial fill with agitation (other surface/volume ratio): [*Describe ratio used*]

3326  Other: [*Summarize approach*]

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<sup>242</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>243</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>244</sup> *Ibid*

<sup>245</sup> *Ibid*

<sup>246</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with ASCA Summary Test Report.

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

3327  
3328

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***Contains Nonbinding Recommendations***

***Draft – Not for Implementation***

3329 **Test Article and Extract Appearance:**

3330  The test article and extract DID NOT change color, and the extract DID NOT appear turbid or  
3331 have particles.

3332  There were changes in color/turbidity or particles in the test article and/or extract OR there  
3333 was swelling/degradation of the test article.<sup>247</sup>

3334

*Describe changes in the test article and/or extract and, if applicable, provide justification to support why changes in the test article and/extract are acceptable.*

3335 **ASCA Test Method SOP #:** [#####-ASCAMLA Agar(date/version)]

3336  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or

3337  Test was conducted per the above protocol and 21 CFR 58, with the following  
3338 deviations/amendments.<sup>248</sup>

*Description of deviations/amendments*

3339

3340

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<sup>247</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>248</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

**Contains Nonbinding Recommendations**

**Draft – Not for Implementation**

3341 **Results:**

3342 **Test Article Extract and Control:**

3343 • **Negative (Vehicle) Control:**

Negative Control (Extraction Vehicle)	Treatment Dose
[e.g., RPMI medium without serum]	[e.g., undiluted RPMI medium supplemented with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]
[e.g., DMSO vehicle]	[e.g., 1% (v/v) dilution of DMSO vehicle in RPMI medium with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]

3344

3345 • **Test Article Extract:**

Test Article Extract	Treatment Dose
[e.g., RPMI medium extract]	[e.g., undiluted RPMI medium extract supplemented with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]
[e.g., DMSO extract]	[e.g., 1% (v/v) dilution of DMSO extract in RPMI medium supplemented with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]

3346

3347 • **Positive Control:**

Positive Control (without S9), 4 hr	Final Concentration (e.g., µg/mL)
[e.g., methyl methane sulfonate (MMS)]	[e.g., 10 µg/mL]
[e.g., MMS]	[e.g., 20 µg/mL]

3348

Positive Control (with S9), 4 hr	Final Concentration (e.g., µg/mL)
[e.g., Cyclophosphamide (CP)]	[e.g., 3 µg/mL]
[e.g., CP]	[e.g., 5 µg/mL]

3349

Positive Control (without S9), 24 hr	Final Concentration (e.g., µg/mL)
[e.g., MMS]	[e.g., 5 µg/mL]
[e.g., MMS]	[e.g., 10 µg/mL]

3350

3351 **Cell Density:**

Suspension Growth Time Point	Initial Cell Density/Adjusted Cell Density
Day 0 (initial treatment day)	(e.g., 3×10 <sup>5</sup> cells/mL)
Day 1 (one day after treatment)	
Day 2 (two days after treatment)	
Day 3 (three days after treatment)	

3352

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3354

**Table 1: Suspension Growth Data**

Suspension Growth Data (4-hour treatment without S9)						
Treatment Group	°Replicate	Cell Density ( $\times 10^5$ cells/mL)		Suspension Growth ( $^{\circ}$ SG)	Relative Suspension Growth ( $^{\dagger}$ RSG)	
		Day 1	Day 2			
*Polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Non-polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Polar Test Extract	Rep. 1					
	Rep. 2					
*Non-polar Test extract	Rep. 1					
	Rep. 2					
*Positive Control	Rep. 1					
	Rep. 2					
Suspension Growth Data (4-hour treatment with S9)						
Treatment Group	°Replicate	Cell Density ( $\times 10^5$ cells/mL)		Suspension Growth ( $^{\circ}$ SG)	Relative Suspension Growth ( $^{\dagger}$ RSG)	
		Day 1	Day 2			
*Polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Non-polar vehicle control	Rep. 1				N/A	
	Rep. 2				N/A	
*Polar test extract	Rep. 1					
	Rep. 2					
*Non-polar Test Extract	Rep. 1					
	Rep. 2					
*Positive Control	Rep. 1					
	Rep. 2					
Suspension Growth Data (24-hour treatment with S9)						
Treatment	°Replicate	Cell Density ( $\times 10^5$ cells/mL)			Suspension Growth ( $^{\circ}$ SG)	Relative Suspension Growth ( $^{\dagger}$ RSG)
		Day 1	Day 2	Day 3		
*Polar Vehicle Control	Rep. 1					N/A
	Rep. 2					N/A
*Non-polar Vehicle Control	Rep. 1					N/A
	Rep. 2					N/A
*Polar Test Extract	Rep. 1					
	Rep. 2					
*Non-polar Test Extract	Rep. 1					
	Rep. 2					
*Positive Control	Rep. 1					

3355

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3357 \*Specify polar vehicle control (e.g., RPMI medium without serum), non-polar vehicle control  
3358 (e.g., DMSO), polar test extract (e.g., RPMI medium test article extract), non-polar test extract  
3359 (e.g., DMSO test article extract), and positive control in the result table. For test article and  
3360 positive controls, two or more concentrations may be used. If so, include a separate row for each  
3361 concentration.

3362 °At least 2 replicates should be used for each test article and control.

3363  ${}^{\circ}SG_{4 \text{ hr treatment}} = \frac{\text{Day 1 cell density}}{\text{Initial cell density}} \times \frac{\text{Day 2 cell density}}{\text{Adjusted cell density}}$

3364  $\diamond SG_{24 \text{ hr treatment}} = \frac{\text{Day 1 cell density}}{\text{Initial cell density}} \times \frac{\text{Day 2 cell density}}{\text{Adjusted cell density}} \times \frac{\text{Day 3 cell density}}{\text{Adjusted cell density}}$

3365  $\dagger RSG = \frac{SG_{\text{test article}}}{SG_{\text{control article}}} \times 100\%$

3366

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**Table 2: Viable Cell Plate (i.e., VC Plate) Colony Count Data**

VC Plate Colony Count Data (4-hour treatment without S9)								
Treatment	°Replicate	Colony Count			Total Colony Count	Cloning Efficiency <sup>□</sup> CE <sub>VC plate</sub> (%)	Relative Cloning Efficiency <sup>‡</sup> RCE (%)	Relative Total Growth <sup>◇</sup> RTG (%)
		Plate 1	Plate 2	Plate 3				
*Polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Non-polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Polar Test Extract	Rep. 1							
	Rep. 2							
*Non-polar Test extract	Rep. 1							
	Rep. 2							
*Positive Control	Rep. 1							
	Rep. 2							

3370

VC Plate Colony Count Data (4-hour treatment with S9)								
Treatment	°Replicate	Colony Count			Total Colony Count	<sup>□</sup> CE <sub>VC plate</sub> (%)	<sup>‡</sup> RCE (%)	<sup>◇</sup> RTG (%)
		Plate 1	Plate 2	Plate 3				
*Polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Non-polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Polar Test Extract	Rep. 1							
	Rep. 2							
*Non-polar Test extract	Rep. 1							
	Rep. 2							
*Positive Control	Rep. 1							

3371



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**Draft – Not for Implementation**

3372

VC Plate Colony Count Data (24-hour treatment without S9)								
Treatment	°Replicate	Colony Count			Total Colony Count	<sup>□</sup> CE <sub>VC plate</sub> (%)	†RCE (%)	◇RTG (%)
		Plate 1	Plate 2	Plate 3				
*Polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Non-polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Polar Test Extract	Rep. 1							
	Rep. 2							
*Non-polar Test Extract	Rep. 1							
	Rep. 2							
*Positive Control	Rep. 1							

3373

3374

3375 \*Specify polar vehicle control (e.g., RPMI medium without serum), non-polar vehicle control (e.g., DMSO), polar test extract (e.g., RPMI medium test article extract), non-polar test extract (e.g., DMSO test article extract), and positive control in the result table. For  
3376 test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.  
3377

3378 °At least 2 replicates should be used for each test article and control.

3379

3380  $\diamond RTG = RSG \times RCE$

3381 
$${}^{\square}CE_{VC\ plate} = \frac{Total\ Colony\ Count/3}{Initial\ number\ of\ cells\ seeded\ in\ VC\ plate} \times 100\%$$

3382

3383 
$$\dagger RCE = \frac{CE_{test\ article}}{CE_{control\ article}} \times 100\%$$

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

**Table 3: Selective Plate (i.e., TFT Plate) Colony Count Data**

3384  
3385

TFT Plate Colony Count Data (4-hour treatment without S9)														
Treatment	Replicate	Colony Counts						<sup>*</sup> N <sub>small</sub>	<sup>*</sup> N <sub>total</sub>	◊CE <sub>TFT</sub>	†MF	°scMF	IMF	‡scIMF
		Plate 1		Plate 2		Plate 3								
		<sup>*</sup> S	<sup>*</sup> L	<sup>*</sup> S	<sup>*</sup> L	<sup>*</sup> S	<sup>*</sup> L							
*Polar Vehicle Control	Rep. 1												N/A	N/A
	Rep. 2													
*Non-polar Vehicle Control	Rep. 1												N/A	N/A
	Rep. 2													
*Polar Test Extract	Rep. 1													
	Rep. 2													
*Non-polar Test Extract	Rep. 1													
	Rep. 2													
*Positive Control	Rep. 1													
	Rep. 2													

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3388

TFT Plate Colony Count Data (4-hour treatment with S9)														
Treatment	Replicate	Colony Counts						<sup>x</sup> N <sub>small</sub>	<sup>x</sup> N <sub>total</sub>	◊CETFT	†MF	<sup>a</sup> scMF	·IMF	‡scIMF
		Plate 1		Plate 2		Plate 3								
		<sup>x</sup> S	<sup>x</sup> L	<sup>x</sup> S	<sup>x</sup> L	<sup>x</sup> S	<sup>x</sup> L							
*Polar Vehicle Control	Rep. 1												N/A	N/A
	Rep. 2													
*Non-polar Vehicle Control	Rep. 1												N/A	N/A
	Rep. 2													
*Polar Test Extract	Rep. 1													
	Rep. 2													
*Non-polar Test Extract	Rep. 1													
	Rep. 2													
*Positive Control	Rep. 1													
	Rep. 2													

3389

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*Draft – Not for Implementation*

TFT Plate Colony Count Data (24-hour treatment without S9)														
Treatment	Replicate	Colony Counts						<sup>*</sup> N <sub>small</sub>	<sup>*</sup> N <sub>total</sub>	◊CE <sub>TFT</sub>	†MF	<sup>¶</sup> scMF	·IMF	‡scIMF
		Plate 1		Plate 2		Plate 3								
		<sup>*</sup> S	<sup>*</sup> L	<sup>*</sup> S	<sup>*</sup> L	<sup>*</sup> S	<sup>*</sup> L							
*Polar Vehicle Control	Rep. 1												N/A	N/A
	Rep. 2													
*Non-polar Vehicle Control	Rep. 1												N/A	N/A
	Rep. 2													
*Polar Test Extract	Rep. 1													
	Rep. 2													
*Non-polar Test Extract	Rep. 1													
	Rep. 2													
*Positive Control	Rep. 1													
	Rep. 2													

3391  
3392

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*Draft – Not for Implementation*

3393 \*Specify polar vehicle control (e.g., RPMI medium without serum), non-polar vehicle control (e.g., DMSO), polar test extract (e.g.,  
3394 RPMI medium test article extract), non-polar test extract (e.g., DMSO test article extract), and positive control (e.g., MMS) in the  
3395 result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each  
3396 concentration.

3397 °At least 2 replicates should be used for each test article and control.

3398 ×S: small colony; L: large colony; N<sub>small</sub>: total small colony count; N<sub>total</sub>: total colony count.

3399 ◊  $CE_{TFT\ plate} = \text{Cloning Efficiency of TFT plate} = \frac{\text{Total Colony Count}/3}{\text{Initial Number of Cells Seeded in TFT Plate}}$

3400 †  $MF = \text{Mutant Frequency} = \frac{CE_{TFT\ plate}}{CE_{VC\ plate}} \times 10^{-6}$

3401 ◻  $scMF = \text{Small Colony Mutant Frequency} = MF \times \frac{\text{Total Small Colony Count}}{\text{Total Colony Count}} \times 100\%$

3402 ·  $IMF = \text{Induced Mutant Frequency} = MF_{\text{test article or positive control}} - \text{Mean } MF_{\text{negative control}}$

3403 ‡  $scIMF = \text{Small Colony Induced Mutant Frequency} = scMF_{\text{test article or positive control}} - \text{Mean } scMF_{\text{negative control}}$

3404 ‡scIMF data are needed for positive controls and test articles if test articles demonstrate a positive response.

3405

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3406 **I confirm that the following test validity criteria are met<sup>249</sup>:**

3407

3408  The negative control meets all following criteria:

3409 

- Mutant Frequency (MF): 35 – 140 per 10<sup>6</sup> cells

3410 

- Cloning Efficiency (CE): 65 – 120%

3411 

- Suspension Growth (SG): 8-32 fold (3-4 hour treatment), 32-180 fold (24-hour treatment)

3413  For both 4-hr (with and without S9) and 24-hr (with S9) assays, the positive controls meet one or more of the following criteria:

3415 

- The positive controls demonstrated an absolute increase in total MF above the spontaneous background MF, and this increase in MF [i.e., the induced MF (IMF)] is at least 300 X 10<sup>-6</sup>. In addition, the small colony IMF is at least 40% of the total IMF.

3418 

- The positive controls demonstrated an increase in the small colony MF above the concurrent negative control. In addition, the increase in the small colony MF (i.e., the small colony IMF) for the positive controls is at least 150 X10<sup>-6</sup>.

3421  The relative total growth (RTG) of cells treated with the positive controls and test article extracts are 10% or greater (i.e., RTG ≥ 10%)

### 3423 Overall Results:

3424

3425  The test article demonstrated a negative response. The results are negative if under all experimental conditions (i.e., 4-hr treatment with and without S9 fraction, 24-hr treatment without S9 fraction), the induced mutant frequency (IMF) in all test article extracts does not exceed the Global Evaluation Factor (GEF)<sup>250</sup> of 90 x 10<sup>-6</sup>.

3429  The test article demonstrated a positive response<sup>251</sup>. The results are positive if under any experimental condition (i.e., 4-hr treatment with and without S9 fraction, 24-hr treatment without S9 fraction), the IMF in any test article extract exceeds the Global Evaluation Factor (GEF) of 90 x 10<sup>-6</sup> AND the RTG of cells treated with the test article extracts is 10% or greater.

3433  The test article demonstrated an equivocal response (i.e., elevated mutant frequency above the concurrent negative control but does not meet the criteria for a positive response)<sup>252</sup>.

3434

*Provide justification to support why an equivocal response is acceptable and repeat testing is not needed. Alternatively, provide repeat test data and explain the findings.*

<sup>249</sup> If any of the test validity criteria are not met, retesting should be conducted and the complete test report should be included with ASCA Summary Test Report.

<sup>250</sup> Global Evaluation Factor (GEF) value is based on the analysis of the distribution of the negative control mutant frequency data from participating laboratories in the International Workshop for Genotoxicity Testing (IWGT). See OECD 490 Guidelines for the Testing of Chemicals – In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene.

<sup>251</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>252</sup> *Ibid*

*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

3435

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*Contains Nonbinding Recommendations*

*Draft – Not for Implementation*

3436 I confirm that:

3437

3438  The above summary information includes all original and any retest data; and

3439  I have checked that there are no differences between the complete test report and this ASCA

3440 summary test report.

3441 Name: [TYPED NAME POSITION]

Date

3442

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*Draft – Not for Implementation*

3443 **C. Example ASCA Summary Test Report: In Vitro Mouse**  
3444 **Lymphoma Test (ISO 10993-3 and OECD 490) —Microwell**  
3445 **Method**

3446 **Administrative Information**

- 3447 1. Testing Laboratory Name:  
3448 2. ASCA Testing Laboratory Identification Number:  
3449 3. Testing Location(s):  
3450 4. Testing Date(s):  
3451 5. *ASCA Accreditation* Status on the Date(s) of Testing:  
3452  Standard (and particular test method) was **NOT** in testing laboratory's scope of *ASCA*  
3453 *Accreditation*<sup>253</sup>  
3454  Standard (and particular test method) was in testing laboratory's scope of *ASCA*  
3455 *Accreditation*  
3456  *ASCA Accreditation* was not suspended  
3457  *ASCA Accreditation* was suspended

*Description of reasons for suspension and their impact on testing results.*

3458  
3459 **ASCA Test Article Prep SOP#:** *[ASCATAPrep(date/version)]*

- 3460  Test Article was prepared per the above protocol (no deviations/amendments); or  
3461  Test Article was prepared per the above protocol, with the following  
3462 deviations/amendments<sup>254</sup> (e.g., filtering, extract manipulation, pH adjustment):

*Description of deviations/amendments*

3463 **Extraction Solvent:**

- 3464  RPMI medium (without serum)  
3465  RPMI medium (with serum) *[Specify serum concentration per SOP and provide*  
3466 *justification (e.g., all other non-polar solvents are not compatible with test article for*  
3467 *extraction)]*  
3468  0.9% Saline *[Provide justification for using 0.9% Saline instead of RPMI medium without*  
3469 *serum]*  
3470  DMSO  
3471  PEG 400 *[Provide justification for using PEG instead of DMSO (e.g., documentation of*  
3472 *device degradation in DMSO)]*

<sup>253</sup> See FDA's guidance [Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#) for information regarding supplemental documentation necessary to support FDA-recognized consensus standards that are not in a testing laboratory's scope of *ASCA Accreditation*.

<sup>254</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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3473  Ethanol [*Provide justification for using ethanol instead of DMSO (e.g., documentation of*  
3474 *device degradation in DMSO)*]

3475  Other:<sup>255</sup> [*Describe*]

#### 3476 **Extraction Ratio:**

3477  6 cm<sup>2</sup>/ml (<0.5 mm thick)\*

3478  3 cm<sup>2</sup>/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)\*

3479  1.25 cm<sup>2</sup>/ml (elastomers > 1.0 mm thick)\*

3480  0.2 g/ml (for powder devices)

3481  0.2 g/ml (for non-powder devices if mass/volume ratio results in equivalent or more test  
3482 article as compared to surface/volume ratio)

3483 \*For absorbent device: [*Specify surface area of test article and the total volume of extraction*  
3484 *vehicle used taking into account the additional volume from absorbency determination)*]

3485  Other:<sup>256</sup> [*Describe*]

3486

#### 3487 **Extraction Conditions:**

3488  37°C, 72 h

3489  50°C, 72 h

3490  70°C, 24 h

3491  121°C, 1 h

3492  Other:<sup>257</sup> [*Describe*]

3493

#### 3494 **Agitation During Extraction:**

3495  Extraction with continuous agitation or circulation

3496  Extraction under static conditions or intermittent agitation<sup>258</sup>: [*Describe and provide*  
3497 *justification*] \_\_\_\_\_

3498

#### 3499 **Fluid Path Extractions:**

3500  For fluid path devices or components (where fluids contact the channels in the device or  
3501 component, and then the fluid enters the body), the extraction was conducted using protocols  
3502 specific to fluid path, with the following approach:<sup>259</sup>

3503  Complete fill with agitation

3504  Partial fill with agitation (ISO 10993-12 surface/volume ratio)

3505  Partial fill with agitation (other surface/volume ratio): [*Describe ratio used*]

3506  Other: [*Summarize approach*]

3507

3508

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<sup>255</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>256</sup> *Ibid*

<sup>257</sup> *Ibid*

<sup>258</sup> *Ibid*

<sup>259</sup> The applicability of various approaches (e.g., partial fill versus complete fill) for a particular device may impact whether or not a complete test report should be included with the ASCA Summary Test Report.

***Contains Nonbinding Recommendations***

***Draft – Not for Implementation***

3509 **Test Article and Extract Appearance:**

3510  The test article and extract DID NOT change color, and the extract DID NOT appear turbid or  
3511 have particles.

3512  There were changes in color/turbidity or particles in the test article and/or extract OR there  
3513 was swelling/degradation of the test article.<sup>260</sup>

3514

*Describe changes in the test article and/or extract and, if applicable, provide justification to support why changes in the test article and/extract are acceptable.*

3515

3516 **ASCA Test Method SOP #:** [#####-ASCAMLAMicrowell(date/version)]

3517  Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or

3518  Test was conducted per the above protocol and 21 CFR 58, with the following  
3519 deviations/amendments.<sup>261</sup>

*Description of deviations/amendments*

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<sup>260</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>261</sup> Since deviations/amendments were noted, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

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3522 **Results:**

3523 **Test Article Extract and Control Dose:**

3524 a. **Negative (Vehicle) Control:**

<b>Negative Control (Extraction Vehicle)</b>	<b>Treatment Dose</b>
[e.g., RPMI medium without serum]	[e.g., undiluted RPMI medium supplemented with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]
[e.g., DMSO vehicle]	[e.g., 1% (v/v) dilution of DMSO vehicle in RPMI medium with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]

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3526 b. **Test Article Extract:**

<b>Test Article Extract</b>	<b>Treatment Dose</b>
[e.g., RPMI medium extract]	[e.g., undiluted RPMI medium extract (100%) supplemented with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]
[e.g., DMSO extract]	[e.g., 1% (v/v) dilution of DMSO extract in RPMI medium supplemented with 5% serum for 4-hr treatment and 10% serum for 24-hr treatment]

3527

3528 c. **Positive Control:**

<b>Positive Control (without S9), 4 hr</b>	<b>Final Concentration (e.g., µg/mL)</b>
[e.g., methyl methane sulfonate (MMS)]	[e.g., 10 µg/mL]
[e.g., MMS]	[e.g., 20 µg/mL]

3529

<b>Positive Control (with S9), 4 hr</b>	<b>Final Concentration (e.g., µg/mL)</b>
[e.g., Cyclophosphamide (CP)]	[e.g., 3 µg/mL]
[e.g., CP]	[e.g., 5 µg/mL]

3530

<b>Positive Control (without S9), 24 hr</b>	<b>Final Concentration (e.g., µg/mL)</b>
[e.g., MMS]	[e.g., 5 µg/mL]
[e.g., MMS]	[e.g., 10 µg/mL]

3531

3532 **Cell Density:**

<b>Suspension Growth Time Point</b>	<b>Cell Density/Adjusted Cell Density</b>
Day 0 (initial treatment day)	(e.g., $3 \times 10^5$ cells/mL)
Day 1 (after one day of suspension growth)	
Day 2 (after two days of suspension growth)	
Day 3 (after three days of suspension growth)	

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**Table 1: Suspension Growth Data**

Suspension Growth Data (4-hour treatment without S9)						
Treatment Group	°Replicate	Cell Density ( $\times 10^5$ cells/mL)			Suspension Growth ( $^{\circ}$ SG)	Relative Suspension Growth ( $^{\dagger}$ RSG)
		Day 1	Day 2			
*Polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Non-polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Polar Test Extract	Rep. 1					
	Rep. 2					
*Non-polar Test extract	Rep. 1					
	Rep. 2					
*Positive Control	Rep. 1					
	Rep. 2					
Suspension Growth Data (4-hour treatment with S9)						
Treatment Group	°Replicate	Cell Density ( $\times 10^5$ cells/mL)			Suspension Growth ( $^{\circ}$ SG)	Relative Suspension Growth ( $^{\dagger}$ RSG)
		Day 1	Day 2			
*Polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Non-polar vehicle control	Rep. 1				N/A	
	Rep. 2				N/A	
*Polar test extract	Rep. 1					
	Rep. 2					
*Non-polar Test Extract	Rep. 1					
	Rep. 2					
*Positive Control	Rep. 1					
	Rep. 2					
Suspension Growth Data (24-hour treatment with S9)						
Treatment	°Replicate	Cell Density ( $\times 10^5$ cells/mL)			Suspension Growth ( $^{\circ}$ SG)	Relative Suspension Growth ( $^{\dagger}$ RSG)
		Day 1	Day 2	Day 3		
*Polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Non-polar Vehicle Control	Rep. 1				N/A	
	Rep. 2				N/A	
*Polar Test Extract	Rep. 1					
	Rep. 2					
*Non-polar Test Extract	Rep. 1					
	Rep. 2					
*Positive Control	Rep. 1					

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\*Specify polar vehicle control (e.g., RPMI medium without serum), non-polar vehicle control (e.g., DMSO), polar test extract (e.g., RPMI medium test article extract), non-polar test extract

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3539 (e.g., DMSO test article extract), and positive control (e.g., MMS) in the result table. For test  
3540 articles and positive controls, two or more concentrations may be used. If so, include a separate  
3541 row for each concentration.

3542 °At least 2 replicates should be used for each test article and control.

3543 <sup>□</sup> $SG_{4\text{ hr treatment}} = \frac{\text{Day 1 cell density}}{\text{Initial cell density}} \times \frac{\text{Day 2 cell density}}{\text{Adjusted cell density}}$

3544 <sup>◇</sup> $SG_{24\text{ hr treatment}} = \frac{\text{Day 1 cell density}}{\text{Initial cell density}} \times \frac{\text{Day 2 cell density}}{\text{Adjusted cell density}} \times \frac{\text{Day 3 cell density}}{\text{Adjusted cell density}}$

3545 <sup>†</sup> $RSG = \frac{SG_{\text{test article}}}{SG_{\text{control article}}} \times 100\%$

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**Table 2: Viable Cell Plate (i.e., VC Plate) Colony Count Data**

VC Plate Colony Count Data (4-hour treatment without S9)								
Treatment	°Replicate	Colony Counts		×N <sub>TW</sub>	×N <sub>EW</sub>	Cloning Efficiency °CE <sub>VC plate</sub> (%)	Relative Cloning Efficiency †RCE (%)	Relative Total Growth ◇RTG (%)
		Plate 1	Plate 2					
*Polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Non-polar Vehicle Control	Rep. 1						N/A	N/A
	Rep. 2						N/A	N/A
*Polar Test Extract	Rep. 1							
	Rep. 2							
*Non-polar Test extract	Rep. 1							
	Rep. 2							
Positive Control	Rep. 1							
	Rep. 2							

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VC Plate Colony Count Data (4-hour treatment with S9)								
Treatment	°Replicate	Colony Counts		×N <sub>TW</sub>	×N <sub>EW</sub>	°CE <sub>VC plate</sub> (%)	†RCE (%)	◇RTG (%)
		Plate 1	Plate 2					
*Polar Vehicle Control	Rep. 1							N/A
	Rep. 2							N/A
*Non-polar Vehicle Control	Rep. 1							N/A
	Rep. 2							N/A
*Polar Test Extract	Rep. 1							
	Rep. 2							
*Non-polar Test extract	Rep. 1							
	Rep. 2							
Positive Control	Rep. 1							



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VC Plate Colony Count Data (24-hour treatment without S9)								
Treatment	°Rep.	Colony Counts		×N <sub>TW</sub>	×N <sub>EW</sub>	▫CE <sub>VC plate</sub> (%)	†RCE (%)	◇RTG(%)
		Plate 1	Plate 2					
*Polar Vehicle Control	Rep. 1							N/A
	Rep. 2							N/A
*Non-polar Vehicle Control	Rep. 1							N/A
	Rep. 2							N/A
*Polar Test Extract	Rep. 1							
	Rep. 2							
*Non-polar Test extract	Rep. 1							
	Rep. 2							
Positive Control	Rep. 1							
	Rep.2							

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3551 \*Specify polar vehicle control (e.g., RPMI medium without serum), non-polar vehicle control (e.g., DMSO), polar test extract (e.g.,  
 3552 RPMI medium test article extract), non-polar test extract (e.g., DMSO test article extract), and positive control (e.g., MMS) in the  
 3553 result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each  
 3554 concentration.

3555 °Rep.=replicate. At least 2 replicates should be used for each test article and control.

3556 × N<sub>TW</sub>=total number of wells with colonies, N<sub>EW</sub>= total number of empty wells.

3557 
$$\text{▫}CE_{VC \text{ plate}} = \frac{-\ln\left(\frac{N_{EW}}{2 \times 96}\right)}{\text{Initial number of cells seeded in VC plate}}$$

3558

3559 
$$\text{†} RCE = \text{Relative Colonizing Efficiency} = \frac{CE_{\text{test article}}}{CE_{\text{control article}}} \times 100\%$$

3560 
$$\text{◇} RTG = RSG \times RCE$$

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**Table 3: Selective Plate (i.e., TFT Plate) Colony Count Data**

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TFT Plate Colony Count Data (4-hour treatment without S9)																	
Treatment	°Rep.	Colony Counts								*N <sub>TW</sub>	*N <sub>S</sub>	*N <sub>EW</sub>	◇CE <sub>TFT</sub>	†MF	°scMF	·IMF	‡scIMF
		Plate 1		Plate 2		Plate 3		Plate 4									
		*S	*L	*S	*L	*S	*L	*S	*L								
*Polar Vehicle Control	Rep. 1															N/A	N/A
	Rep. 2																
*Non-polar Vehicle Control	Rep. 1															N/A	N/A
	Rep. 2																
*Polar Test Extract	Rep. 1																N/A
	Rep. 2																
*Non-polar Test Extract	Rep. 1																N/A
	Rep. 2																
*Positive Control	Rep. 1																
	Rep.2																

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TFT Plate Colony Count Data (4-hour treatment with S9)																	
Treatment	°Rep.	Colony Counts								×NTW	×Ns	×NEW	◇CE <sub>TFT</sub>	†MF	°scMF	IMF	‡scIMF
		Plate 1		Plate 2		Plate 3		Plate 4									
		×S	×L	×S	×L	×S	×L	×S	×L								
*Polar Vehicle Control	Rep. 1															N/A	N/A
	Rep. 2																
*Non-polar Vehicle Control	Rep. 1															N/A	N/A
	Rep. 2																
*Polar Test Extract	Rep. 1																
	Rep. 2																
*Non-polar Test Extract	Rep. 1																
	Rep. 2																
*Positive Control	Rep. 1																
	Rep. 2																

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TFT Plate Colony Count Data (24-hour treatment without S9)																	
Treatment	Rep.	Colony Counts								<sup>x</sup> N <sub>TW</sub>	<sup>x</sup> N <sub>S</sub>	<sup>x</sup> N <sub>EW</sub>	◊CE <sub>TFT</sub>	†MF	<sup>o</sup> scMF	IMF	‡scIMF
		Plate 1		Plate 2		Plate 3		Plate 4									
		<sup>x</sup> S	<sup>x</sup> L	<sup>x</sup> S	<sup>x</sup> L	<sup>x</sup> S	<sup>x</sup> L	<sup>x</sup> S	<sup>x</sup> L								
*Polar Vehicle Control	Rep. 1															N/A	N/A
	Rep. 2																
*Non-polar Vehicle Control	Rep. 1															N/A	N/A
	Rep. 2																
*Polar Test Extract	Rep. 1																
	Rep. 2																
*Non-polar Test Extract	Rep. 1																
	Rep. 2																
*Positive Control	Rep. 1																
	Rep. 2																

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3571 <sup>x</sup>S=small, L=Large, N<sub>TW</sub>=total number of wells with colonies, N<sub>EW</sub>= total number of empty wells, N<sub>s</sub>=total number of wells with  
3572 small colonies.

3573 <sup>o</sup>Rep.=replicate. At least 2 replicates should be used for each test article and control.

3574 <sup>\*</sup>Specify polar vehicle control (e.g., RPMI medium without serum), non-polar vehicle control (e.g., DMSO), polar test extract (e.g.,  
3575 RPMI medium test article extract), non-polar test extract (e.g., DMSO test article extract), and positive control (e.g., MMS) in the  
3576 result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each  
3577 concentration.

3578  $\diamond CE_{\text{TFT plate}} = \frac{-\ln \frac{N_{EW}}{4 \times 96}}{\text{Initial number of cells seeded per well in TFT plate}}$

3579

3580  $\dagger \text{MF} = \text{Mutant Frequency} = \text{MF} = \frac{CE_{\text{TFT plate}}}{CE_{\text{VC plate}}} \times 10^{-6}$

3581  $\text{scMF} = \text{Small Colony Mutant Frequency} = \text{MF} \times \frac{N_s}{N_{TW}} \times 100\%$

3582  $\text{IMF} = \text{Induced Mutant Frequency} = \text{MF}_{\text{test article or positive control}} - \text{Mean MF}_{\text{negative control}}$

3583

3584  $\ddagger \text{scIMF} = \text{Small Colony Mutant Frequency} = \text{scMF}_{\text{test article or positive control}} - \text{Mean scMF}_{\text{negative control}}$

3585  $\ddagger \text{scIMF}$  data are needed for positive controls and test articles if test articles demonstrate a positive response.

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3586 **I confirm that the following test validity criteria are met<sup>262</sup>:**

3587

3588  The negative control meets the following criteria:

3589 

- Mutant Frequency (MF): 50 – 170 per 10<sup>6</sup> cells

3590 

- Cloning Efficiency (CE): 65 – 120%

3591 

- Suspension Growth (SG): 8-32 fold (3-4 hour treatment), 32-180 fold (24-hour treatment)

3593  For both 4-hr (with and without S9) and 24-hr (without S9) assays, the positive control(s)

3594 meet at least one of the following criteria:

3595  The positive control demonstrated an absolute increase in total MF, that is, an

3596 increase above the spontaneous background MF, and this increase in MF [i.e.,

3597 the induced MF (IMF)] is at least 300 X 10<sup>-6</sup>. In addition, the small colony IMF

3598 is at least 40% of the total IMF.

3599  The positive control demonstrated an increase in the small colony MF above the

3600 concurrent negative control and the increase in the small colony MF (i.e., the

3601 small colony IMF) is at least 150 X10<sup>-6</sup>

3602  The relative total growth (RTG) of cells treated with the positive controls is 10% or

3603 greater (i.e., RTG ≥ 10%)

3604 **Overall Results:**

3605  The test article demonstrated a negative response. The results are negative if under all

3606 experimental conditions (i.e., 4-hr treatment with and without S9 fraction, 24-hr treatment

3607 without S9 fraction), the induced mutant frequency (IMF) in all test article extracts does not

3608 exceed the Global Evaluation Factor (GEF)<sup>263</sup> of 126 x 10<sup>-6</sup>.

3609  The test article demonstrated a positive response<sup>264</sup>. The results are positive if under any

3610 experimental conditions (i.e., 4-hr treatment with and without S9 fraction, 24-hr treatment

3611 without S9 fraction) examined, the IMF in any test article extract exceeds the Global

3612 Evaluation Factor (GEF) of 126 x 10<sup>-6</sup> AND the RTG of cells treated with the test article

3613 extracts is 10% or greater.

3614  The test article demonstrated an equivocal response (i.e., elevated mutant frequency above

3615 the concurrent negative control but does not meet the criteria for a positive response)<sup>265</sup>.

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<sup>262</sup> If any of the test validity criteria are not met, retesting should be conducted and the complete test report should be included with ASCA Summary Test Report.

<sup>263</sup> Global Evaluation Factor (GEF) value is based on the analysis of the distribution of the negative control mutant frequency data from participating laboratories in the International Workshop for Genotoxicity Testing (IWGT). See OECD 490 Guidelines for the Testing of Chemicals – In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene.

<sup>264</sup> In this situation, the complete test report should be included with ASCA Summary Test Report. Test Laboratory/Manufacturer should also provide a rationale to support a regulatory decision.

<sup>265</sup> *Ibid*

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*Provide justification to support why an equivocal response is acceptable and repeat testing is not needed. Alternatively, provide repeat test data and explain the findings.*

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3617 I confirm that:

3618

3619  The above summary information includes all original and any retest data; and

3620  I have checked that there are no differences between the complete test report and this  
3621 ASCA summary test report.

3622 \_\_\_\_\_  
3623 Name: [TYPED NAME POSITION]

Date

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