Contains Nonbinding Recommendations

Draft – Not for Implementation

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Draft Guidance for Industry, Accreditation Bodies, Testing Laboratories, and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.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draft document within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance.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. 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.

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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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Table of Contents

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

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic.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

I. Introduction

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 The ASCA Program is described in FDA's draft guidance The Accreditation Scheme for Conformity Assessment (ASCA) Program.

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

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

II. Scope

This guidance includes the following:

- 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.

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

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[1] 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.[2]

[1] 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.

[2] 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".

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

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

- ASTM F756: *Standard Practice for Assessment of Hemolytic Properties of Materials*
- ASTM F720: *Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test*
- ISO 10993-4: *Biological evaluation of medical devices – Part 4: Selection of tests for interactions with blood*
- ISO 10993-5: *Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity*
- ISO 10993-10: *Biological evaluation of medical devices – Part 10: Tests for skin sensitization*
- ISO 10993-11: *Biological evaluation of medical devices – Part 11: Tests for systemic toxicity*
- USP <151>: *Pyrogen Test*
- ISO 10993-12: *Biological evaluation of medical devices – Part 12: Sample preparation and reference materials*
- ISO 10993-23[4]: *Biological evaluation of medical devices – Part 23: Tests for irritation*
- ISO 10993-2: *Biological evaluation of medical devices — Part 2: Animal welfare requirements*

[3] 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.

[4] Irritation tests are moved from ISO 10993-10 fourth edition and published as ISO 10993-23.

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

FDA-Recognized Consensus Standard	Test method(s)
ISO 10993-4*	SC5b-9 Complement Activation[5] 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[6]	Closed Patch Sensitization
ISO 10993-23[7]	Dermal Irritation, Intracutaneous Reactivity Irritation
ISO 10993-10 and ASTM F720[8]	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

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

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

IV. Assessment and Accreditation of Testing Laboratories

A. Scope of Assessments

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

[5] 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.

[6] 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

[7] *Ibid*

[8] *Ibid*

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

requirements of the FDA-recognized consensus standards and test methods within the laboratory's scope of accreditation.[10]

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

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

This assessment should include:

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

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

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

[11] 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.

B. Competency of Accreditation Body's Technical Assessors

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

i. All technical assessors are knowledgeable in all of the FDA-recognized consensus standards and test methods under the ASCA Program, the ASCA Program specifications in Section IV.E. of this guidance, as well as 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*," and

ii. All technical assessors have[12] a Bachelor's or higher degree in a scientific discipline and one of the following, at a minimum:
   - two years relevant experience in medical device biocompatibility testing,
   - two years relevant experience with in vivo testing, or
   - two years relevant experience with in vitro testing (e.g., cell biology testing).

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

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

C. Accreditation by ASCA-Recognized Accreditation Bodies

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

[12] 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.

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

i) conducts fraudulent activities or has issues with test data integrity,
ii) is non-compliant with or in violation of 21 CFR 58,
iii) copies or paraphrases ASCA specifications or FDA-recognized consensus standards clauses into the controlled documents without detailed procedures on implementation of the ASCA Program specifications and FDA-recognized consensus standards clauses,
iv) lacks document control,
v) lacks evidence demonstrating conformance to all applicable FDA-recognized consensus standards clauses and ASCA specifications in the requested scope of accreditation for ASCA, or
vi) has animal welfare[13] issues (e.g., unresolved animal welfare violations based on a national authority's inspection).

D. Scope of ASCA Accreditation Issued by Accreditation Bodies

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

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

[13] 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

E. ASCA Program Specifications for Biocompatibility Testing of Medical Devices

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

ASCA-recognized accreditation bodies will assess the testing laboratories to the specifications below. In addition, all testing should be conducted considering the recommendations in 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 readability and ease of reference, the numbering and nomenclature (including the term requirements)[14] below correspond to the numbering and nomenclature of clauses/subclauses in ISO/IEC 17025.

ISO/IEC 17025 Clause 4 "General requirements"

4.1 Impartiality

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

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

4.2 Confidentiality

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

[14] 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.

ISO/IEC 17025 Clause 5 "Structural requirements"

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

ISO/IEC 17025 Clause 6 "Resource requirements"

6.1 General

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

6.2 Personnel

a) The testing laboratory will maintain competent technical personnel that are knowledgeable in the appropriate test method(s) for the requested scope of accreditation and have relevant experience and education that meet the criteria as described in Appendix A of this guidance.

b) The testing laboratory's management will be knowledgeable in applicable aspects of the FD&C Act and 21 CFR regulations pertinent to the oversight of medical devices and the criteria set out in ISO/IEC 17025 and ASCA Program specifications. The testing laboratory further will maintain a list of laboratory managers and contact information.

c) The testing laboratory will:

   1) Document and maintain a training program for new and previously trained technical personnel, which will include the proper procedures for applying new/updated test procedures and performing required tests;

   2) Provide new and previously trained technical personnel relevant test-specific requalification training (e.g., cytotoxicity subjective scoring) every 6-12 months, or when test standards or procedures are updated or developed, as well as when responsibilities have changed;

   3) Conduct training on a periodic basis through application of training approaches, such as on-the-job training and formal classroom training, as appropriate;

   4) Document and maintain records of training demonstrating that technical personnel who participate in the conduct of ASCA testing have been trained and evaluated to be competent in the performance of each ASCA test. The training includes the ability to follow test-related standard operating procedures (SOPs) and documentation, and in person hands-on training. Training may also include classroom (or online) training. Testing laboratories further agree to have predefined criteria to qualify that technical personnel (technicians and study directors) can perform assigned tasks related to the tests under the scope of *ASCA Accreditation*, and for when retraining will be needed (e.g., when technicians have not conducted the test or assigned task for a specified period of time, change in assigned activities, when procedures are updated, as a corrective or preventive action in response to non-conformance to procedures), and follow-up actions needed in the case of mock study or proficiency check failures;

5) Establish procedures for periodic internal test lab proficiency checks[15] of technicians (e.g., blind scoring of negative and positive controls for MEM elution assays) for the tests performed under the ASCA Program with subjective analyses; and

6) Maintain records demonstrating trainers have qualifications and at least 2 years' experience (routinely performing each relevant ASCA test) to train the technical personnel (i.e., technicians or trainees) who will perform the ASCA tests.

d) The testing laboratory will have procedures to establish how test and control samples are prepared and training (e.g., training manuals, training records) that includes the following:

1) Procedures for device preparation, including:

i. Cutting samples (if appropriate) including examples of devices that should not be cut and how to handle previously unexposed surfaces when cutting samples as well as documentation (e.g., photographs) of any particle generation prior to extraction;

ii. Determination of device surface area for extraction ratio including how surface area is calculated (e.g., calculation based on dimensional measurement using formula, calculation based on engineering drawings, sponsor provided surface area based on engineering drawings), and the volume required to complete the study;

iii. Use of non-standard surface area approaches (e.g., porous devices),

iv. Exclusion of non-contacting components from extraction;

v. Selection of representative portions for direct contact hemocompatibility studies (i.e., hemolysis, complement activation);

vi. Selection of extraction time and temperature. The following is a list of acceptable extraction times and temperatures:

- (37±1)°Cfor (24±2)h[a]
- (37±1)°Cfor (72±2)h[b]
- (50±2)°Cfor (72±2)h[c]
- (70±2)°Cfor (24±2)h[c]
- (121±2)°Cfor (1±0.1)h[c]

h = hour

Note a: for MEM elution cytotoxicity testing of devices with limited (less than or equal to 24 h) contact.

[15] For hemolysis, complement activation, and material-mediated pyrogenicity testing, there are no ASCA specifications for a periodic proficiency check.

Note b: for MEM elution cytotoxicity testing of devices with prolonged (>24 h to 30 days (d)) or long-term (>30 d) contact.

Note c: for testing (other than MEM elution cytotoxicity) of devices if devices do not contain heat labile or heat sensitive materials (e.g., drugs) or if devices do not contain materials that may have the potential to undergo deformation or material configuration/structural change at such temperature.

vii. Selection of extraction vehicle. Common extraction vehicles for each test method within the ASCA Program are listed in the "Extraction Vehicle" section of Appendices C~K of this guidance; and

viii. Solvent compatibility pre-test between the selected solvent and the test article as well as when solvent compatibility pre-test should be conducted (e.g., when softening or deformation of the device material occurs in the presence of the solvent).

2) Assessment and documentation of changes (e.g., photographs) after extraction to sample (e.g., color changes, integrity, swelling) or extract conditions (e.g., pH, particles/precipitates, color changes, or turbidity);

3) General and/or test-specific follow-up procedures when changes are noted (e.g., extract settling techniques to allow particle-free IV injections);

4) Use of non-standard extraction approaches (e.g., fluid path approaches, approaches for extremely large devices, procedures to maintain contact with extraction vehicle);

5) Handling of extracts prior to testing (e.g., filtration, centrifugation, storage time and temperature); and

6) Extract storage (e.g., used immediately, refrigerated at 2 to 8 ºC for no longer than 24 hours without visible precipitation).

e) In addition, for each specific test method, the testing laboratory agrees to establish a training program (e.g., training manual, training records) to demonstrate how training will be conducted and how technician competency will be evaluated (e.g., for in vivo tests, number of animals used in training technicians and acceptance criteria for task completion). At a minimum, the training program will address the test method-specific ASCA specifications in each associated Appendix (e.g., Appendices C-K).

f) For in vivo studies, as part of the general animal care and observation training, the testing laboratory agrees to establish a training program (e.g., training manual, training records) to demonstrate how training will be conducted and how technician competency will be evaluated (e.g., acceptance criteria). This training program and the procedures related to animal care and handling will address the following, at a minimum:

1) Test-specific animal model selection criteria (e.g., species/strain, age, weight, sex, and source);

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

6.3 Facilities and environmental conditions

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

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

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

6.4 Equipment

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

c) The testing laboratory will ensure that its procedures specify the steps for establishing calibration intervals for each type or item of equipment, and specify criteria, steps, and approvals for extending the calibration interval of an instrument.

d) The testing laboratory will have procedures to examine the effects of equipment operation outside the equipment tolerances or study specified limits (e.g., temperature excursions) on test results. The procedures identify the personnel responsible for such examination of the equipment (e.g., technicians) and determination of acceptability with respect to test validity (e.g., study directors/toxicologists), specify their responsibilities, and provide the steps for determining if the equipment variation would impact the study results, including:

1) Determining whether the effects are unacceptable (including the accept/reject criteria),
2) Identifying the conducted tests affected,
3) Analyzing the results impacted for these particular tests, and
4) Determining whether retesting is required.

6.5 Metrological traceability

a) Testing laboratories agree to use specified methods and/or standards that clearly describe the following:

1) Calibration to three decimal places for spectrophotometer absorbance readings for hemolysis and complement activation, and
2) Particle ranges for calibration of coulter counter use for cell counting.

b) If test-specified positive, negative, and/or reference controls are no longer able to distinguish between positive and negative responses, the testing laboratory will have procedures to qualify new lots or new suppliers of the controls. The procedure should address how each test-specific control (positive/negative/reagent, if applicable) will be qualified for each test method. For example, material specifications for purchasing controls could be established based on Certificate of Analyses (CoAs) so that only controls that meet the pre-established specifications (e.g., purity, reagent grade, appearance) can be used for testing. If verification testing is used to qualify the controls, testing laboratories need to establish procedures and document test methods used to qualify the controls. In addition, the frequency of qualification testing, acceptance criteria, and expiration date for the controls should be specified in the procedure. Appendix L in this guidance includes a table for the controls and reagents that could impact the validity of a test, and for which purchase control and/or verification testing specifications need to be established.

c) The testing laboratory agrees that controls (positive/negative/reagent, if applicable) will meet assay-specific acceptance criteria. For example, testing laboratory will establish the procedure to monitor the performance of positive and negative control

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

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

6.6 Externally provided products and services

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

ISO/IEC 17025 Clause 7 ("Process requirements")

7.1 Review of requests, tenders and contracts

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

7.2 Selection, verification and validation of methods

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

1) Identifying the personnel responsible for developing, reviewing, and maintaining these documents,
2) Specifying the frequency of review by technical personnel and management
3) Ensuring consistency with applicable standard(s),
4) Ensuring test modifications are reviewed by personnel who are competent to the applicable standard(s), and
5) Identifying and documenting the types of modifications to the test procedures that do not need to be reviewed by FDA for confirmation prior to implementation, if included in the test lab application.

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

i. Changes to sample for retesting to achieve a "passing" result
ii. pH adjustments,

iii. Sample filtration or other extract manipulation,

iv. Removal or modification of documentation associated with color, turbidity or particles in the test extract, or swelling/degradation of the test article

v. Frequency of non-concurrent control testing,

vi. Changes to acceptance criteria outside the validated/qualified laboratory-specific limits (e.g., for complement activation where the standard methods do not specify acceptable limits),

vii. Changes to data calculations and presentation, if applicable (e.g., hemolytic index, irritation index, complement activation plots),

viii. Changes in the criteria for re-challenge or retesting,

ix. Changes in the criteria for reportable adverse clinical observations or animal deaths, and

x. Any unanticipated changes.[16]

c) The testing laboratory agrees that test procedures will include or specify, as appropriate, the following:

1) Unique identification, including title, document number, revision, and effective date,

2) Specific test equipment to use along with their required ratings,

3) Warnings/caution statements to alert the operators of potential hazards,

4) Normal and any unusual ambient conditions (including tolerances) for tests,

5) Test data to be obtained and recorded,

6) Objective acceptance criteria for results including the essential performance required to be maintained,

7) Testing techniques (i.e., test methods) required to ensure consistent results,

8) Instructions on test conduct, including equipment operation, reagent preparation, cell line and animal handling, techniques, preparation of test samples (including instructions for sample traceability during testing, if applicable), conduct of each step of the test, data recording, and scoring assessment procedures,

9) Deviations from the SOP, as well as any equipment deviations and discussion of why deviations will not impact the validity of the study results, and

10) If the procedure is written for both ASCA testing and non-ASCA testing, then the portion of the procedure excluded from ASCA testing should be clearly specified.

[16] 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.

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

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

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

7.3 Sampling

a) The testing laboratory agrees that the procedure(s) for sample preparation will meet the specifications of ISO 10993-12 and 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".[17] The testing laboratory will also establish a training program (e.g., training manuals, training records). The procedures and the training should address the following::

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

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

3) How sample extraction ratio (e.g., 6 cm^2/ml, 3 cm^2/ml) will be selected,

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

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

6) No filtration/centrifugation,

7) No pH/osmolality adjustment,

8) No complete device dissolution during extraction,

[17] 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

9) Documentation of any color changes, turbidity or particles in the extract,

10) How representative portions are proportionally selected for testing, if the test system cannot accommodate all of the direct and indirect tissue contacting device components, to include documentation of what was excluded,

11) How extraction vehicle volume will be determined and documented for absorbent devices (e.g., spongy or porous devices) including details on absorbency capacity determination,

12) How sample extraction ratios will be selected for devices having multiple components with different thicknesses,

13) How components with different types and durations of contact will be separated for sample preparation and testing,

14) Situations when pooled component samples (with same or different types or duration of tissue contact) will be allowed,

15) Inclusion of only tissue contacting components (unless procedure describes how inclusion of non-tissue contacting components will be addressed in determination of extraction ratios),

16) Submersion of large devices completely in extraction vehicle,

17) How extractions will be conducted for devices containing fluid path components (e.g., complete fill, partial fill with agitation (ISO 10993-12 surface/volume ratio), partial fill with agitation (other surface/volume ratio)), and

18) That the following types of devices are excluded for the ASCA Program: devices that require customized[18] sample preparation and/or testing methodologies, absorbable devices, in situ polymerizing devices, liquid devices, creams, gels, hydrogel devices, and devices containing nanomaterials.

7.4 Handling of test or calibration items

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

7.5 Technical records

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

7.6 Evaluation of measurement uncertainty

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

7.7 Ensuring the validity of results

[18] 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.

a) To confirm the validity of the testing methods, any test-specified positive, negative, and/or reference controls allow for distinguishing between positive and negative responses. The testing laboratory agrees that pre-defined criteria for positive/negative/reference control values will meet the test method-specific ASCA specifications in each associated Appendix in this document.

b) Testing laboratories agree to establish procedures for management of unexpected results and non-conformance quality events (e.g., non-conformance to test method SOPs, protocols, and work instructions) encountered during testing and operational activities. General procedures should be established for root cause investigation and analysis to support corrective and preventive actions.

7.8 Reporting of results

a) The testing laboratory agrees that it will have procedures to record all required information in ISO/IEC 17025 for each test conducted, including the following:

1) Test procedure(s) and test standard(s) used,

2) Product or component(s) tested,

3) Test equipment used for testing, measurement, or review (including the equipment's ratings and accuracies, unless otherwise readily available),

4) Date of the test(s). For example, periodic controls may have different test dates,

5) Test report number, including revision number and amendment date, if applicable, and any related sub-contracted test report number(s),

6) Names of the personnel performing the test(s) and the names of all supervisory personnel involved in the study and for biological studies, the signature of the study director and quality assurance unit personnel (i.e., per 21 CFR part 58, Good Laboratory Practices for Nonclinical Laboratory Studies, requirements),

7) The test conditions as specified by the test standard, if applicable, (e.g., required voltage, power, temperature, or humidity for the test),

8) Sample preparation:

i. images of device (or representative portion, if full device is not used) prior to and post sample preparation,

ii. documentation of device components that are sampled, and those that are not sampled, and

iii. use of subdivision/cutting.

9) Extraction conditions, if applicable:

i. extraction vehicle, time, temperature, means of agitation, and test article/vehicle ratio,

ii. storage time and temperature prior to application to the test system, and

iii. images of vehicle post-extraction (color, cloudiness, presence of particulates).

10) Sample manipulation:

i. filtration, centrifugation, dilution, pH adjustment, osmolality adjustment or other deviations from the sampling procedures,

11) Any deviations from the laboratory's ASCA accepted procedures as well as any amendments to the test report,

12) Test results to include:

i. opinions and interpretations included in a test report,

ii. all of the applicable data required by the laboratory's procedures, and

iii. a statement that testing was conducted according to 21 CFR 58 Good Laboratory Practices for Nonclinical Laboratory Studies regulations.

b) The testing laboratory agrees that testing conducted by subcontractors will also comply with the above test report specifications, as applicable, and

c) The testing laboratory agrees that the complete test report and an ASCA Summary Test Report will be submitted to the client at the end of testing activities.

7.9 Complaints

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

7.10 Nonconforming work

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

7.11 Control of data and information management

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

ISO/IEC 17025 Clause 8 ("Management system requirements")

8.1 Options

Regardless of the option selected (i.e., Option A or Option B), the testing laboratory will maintain SOPs and any relevant ASCA test-related 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) applicable to any biological evaluation of medical device standards or test methods.

V. Recommendations for Testing Laboratory Participation in the ASCA Program

A. Demonstration of Competency

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

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

B. Test Plan Development

FDA encourages testing laboratories that participate in the ASCA Program to work with device manufacturers to develop a test plan on what specific biocompatibility assessments will be conducted based on the type and duration of device contact that is consistent with recommendations as described in Attachment A 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*." Testing laboratories are expected to include a general procedure (e.g., a flowchart) in the controlled documents to specify how type and duration of device contact will determine recommended biocompatibility assessment.

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

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

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

A. Cover Letter

FDA's recommendations regarding the content to be included in a cover letter for a premarket submission containing testing results from an ASCA-accredited testing laboratory are provided in FDA's guidance The Accreditation Scheme for Conformity Assessment (ASCA) Program.

B. ASCA Declaration of Conformity (ASCA DOC)

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

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

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

C. Supplemental Documentation

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

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

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

The ASCA Program processes and policies enhance confidence in testing results only when specific test methods and acceptance criteria are used. For example, FDA reviews a copy of SOPs and any relevant ASCA test-related 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) for testing laboratories that apply for a scope of *ASCA Accreditation* that includes biocompatibility testing. This review provides FDA an understanding of how testing is conducted, thereby providing confidence in the competence of ASCA-accredited testing laboratories. Depending on the specific device or intended use, deviations or amendments relative to the testing documentation submitted to FDA during the *ASCA Accreditation* application process[20] may be appropriate. In such cases, FDA recommends a complete test report be included in the premarket submission. FDA also recommends a complete test report be included in the premarket submission for specific circumstances when (based on FDA's review experience) results may indicate a potential concern. These cases are noted below and, in each example, ASCA Summary Test Report (*refer to the test method-specific Appendices of this guidance*).

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

[19] 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"

[20] 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.

- 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, Clause7.3.7), 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.,

per ISO 10993-10:2021, Clause 6.6.6), or the sodium chloride and oil vehicle controls are > Grade 0,
    - if differences in source, strain, treatment methods, or timing of the positive control occurred, and
    - if adverse clinical findings or animal deaths occurred.
- For acute systemic toxicity (ISO 10993-11):
    - if any test or control animals died or had any adverse clinical findings, and
    - if any test animals had a body weight loss greater than 10%.
- For material-mediated pyrogenicity (ISO 10993-11 and USP 151):
    - if any rabbit has a baseline temperature exceeding 39.8°C, or if any rabbit has a temperature rise ≥0.5°C, and
    - if adverse clinical findings or animal deaths occurred.
- For direct and indirect hemolysis (ISO 10993-4 and ASTM F756):
    - if direct contact was used and a diluent other than Magnesium and Calcium Free PBS was used (i.e., per ASTM F756-17, Section 3.1.10),
    - if direct contact was used and an exposure ratio other than those specially called out in the example ASCA Summary Test Report (*refer to Appendix J of this guidance*) was used (i.e., per ASTM F756-17, Section 9.2.1),
    - if the test article red blood cell pellet after centrifugation was visually different in color or size (e.g., larger or very small) compared to the pellet for the negative control,
    - 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 ≥2%, and
    - if the total hemoglobin concentration of the diluted blood is outside of the range of 9-11 mg/ml.
- For SC5b-9 complement activation (ISO 10993-4):
    - if a test medium other than those specifically called out in the example ASCA Summary Test Report (*refer to Appendix K of this guidance*) was used,
    - if exposure ratio or conditions other than those specially called out in the example ASCA Summary Test Report (*refer to Appendix K of this guidance*) were used, and
    - 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.

VII. Paperwork Reduction Act of 1995

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

The time required to complete this information collection is estimated[21] to average 95 hours per response for accreditation bodies and 47 hours for testing laboratories. Send comments regarding this burden estimate or suggestions for reducing this burden to:

FDA PRA Staff,
Office of Operations,
Food and Drug Administration,
PRAStaff@fda.hhs.gov

> 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).

[21] Rounded to the nearest whole number.

Appendix A: Relevant Experience and Educational Requirements[22] for Technical Personnel that Conduct ASCA Tests

Personnel Type	Experience	Education
Technicians performing in vivo tests	Meet one of the following, at a minimum: • 1 year of relevant test experience with each standard test included in the ASCA Program to which technicians are assigned • 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.	Meet one of the following, at a minimum: • Bachelor's or associate degree in relevant science areas to the biocompatibility testing included in the ASCA Program • a high school degree, and at least one of the following laboratory technician accreditations[23]: o Assistant Laboratory Animal Technician (ALAT), o Laboratory Animal Technician (LAT) o Laboratory Animal Technologist (LATG).
Technicians performing in vitro tests	Meet one of the following, at a minimum: • 1 year of relevant test experience with each standard test included in the ASCA Program to which technicians are assigned • 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	Meet one of the following, at a minimum: • Bachelor's or associate degree in relevant science areas to the biocompatibility testing included in the ASCA Program • 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

[22]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.

[23] 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").

Technicians performing any test specific (e.g., for complement activation) sample preparation	Meet one of the following, at a minimum: • 1 year of sample preparation experience with the relevant standard test included in the ASCA Program to which technicians are assigned • demonstrated proficiency through completion of sample preparation for minimally 25 tests to which technicians are assigned	Meet one of the following, at a minimum: • Bachelor's or associate degree in science • 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
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)	Meet one of the following, at a minimum: • 1 year sample preparation experience with any standard test included in the ASCA Program • demonstrated proficiency through completion of sample preparation for minimally 25 of any of the standard tests in the ASCA Program	Meet one of the following, at a minimum: • Bachelor's or associate degree in science • 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.
Study directors	2 years of relevant test experience with each standard test and meet one of the following, at a minimum: • direction of at least 25 studies in each relevant test • management of 25 studies with someone who has directed at least 25 studies in each relevant test	Bachelor's or higher degree in scientific discipline

862

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

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

Responsible Party

Name of entity responsible for DOC: _____

Address of entity responsible for DOC: _____

Product/Device Identification

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

Statement of Conformity

The test results demonstrate that the device is in conformity with the standard(s) listed below:[24]

1. Title of Standard(s)[25]: (e.g., *ISO 10993-10 Third edition 2021-11 Biological evaluation of medical devices – Part 10: Tests for skin sensitization*)___
   - FDA Recognition #(s): *(e.g., 2-296)*
   - Options Selected
     - ☐ Standard(s) included no options
     - ☐ Standard(s) included options

       List of options selected in standards:

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

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

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

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

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

[24] See section 514(c)(3)(A)(i) of the FD&C Act, cited in Section IV.A.(3)(f) of FDA's guidance.

[25] 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.

☐ Closed Patch Sensitization: ISO 10993-10:2021 Clause 6.6
☐ Dermal Irritation: ISO 10993-23:2021 Clause 7.2
☐ Intracutaneous Reactivity Irritation: ISO 10993-23:2021 Clause 7.3
☐ Acute Systemic Toxicity: ISO 10993-11:2017 Clause 5
☐ Material-Mediated Pyrogenicity: ISO 10993-11:2017 Annex G and USP <151>

- Testing Laboratory Name: (*e.g., Testing Laboratory ABC)*
- ASCA Testing Laboratory Identification Number (as applicable): (*e.g., ASCA001)*
- Testing Location(s): (*e.g., 1234 Example Road, Silver Spring, MD 20993)*
- Testing Date(s): (*e.g., Sep 1, 2024 – Sep 15, 2024)*
- *ASCA Accreditation* Status on the Date(s) of Testing:

☐ Standard(s) (and particular test methods) were not in testing laboratory's scope of *ASCA Accreditation*

☐ Standard(s) (and particular test methods) were in testing laboratory's scope of *ASCA Accreditation*

☐ *ASCA Accreditation* was not suspended

☐ *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.*

- Supplemental Documentation (*see Section VI.C. of this guidance for specific recommendations*):

☐ Supplementary documentation is not included

☐ Supplementary documentation is included at the following location within the submission, and I have checked that there are no differences regarding protocol and data between the complete test report and the supplemental documentation: (*e.g., MEM Elution Cytotoxicity (ISO 10993-5)ASCA Summary Test Report located in Appendix A of this premarket submission*)

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[26] (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*

Signature

Printed name: _____

Function within entity responsible for DOC: _____

_____

Signature Date

[26] 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.

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

i. Shaving techniques (e.g., to avoid razor burn),
ii. Application of test samples,
iii. Injection technique and signs to confirm appropriate injection location,
iv. Injection site scheme and marking,
v. Differentiation for source of redness (e.g., true irritation versus possible irritation from shaving),
vi. Clinical observations (e.g., cage side observation, skin site observation, and presence of adverse events), criteria for assessment, data capture, and frequency (e.g., minimum daily),
vii. Evaluation criteria and basis for retest,
viii. Data documentation, calculations, analysis and result interpretation,
ix. Minimally, biannual periodic technician proficiency check of positive response scoring (in live animals at least once annually). The proficiency check procedure should specify, at a minimum, the following:

- positive and negative controls used,
- number of positive and negative controls, number of animals and/or number of images used (if images are used for proficiency check),
- protocol used to conduct the study, and
- pass/fail criteria. For example, comparison of technician and trainer scores and criteria for acceptable level of agreement: for each individual site, and the overall score (e.g., irritant vs. non-irritant).

x. Criteria for technician retraining, if needed.

ISO/IEC 17025 Subclause 7.7(a)

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

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

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

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*[27]

☐ 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[28] (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]*

☐ Other:[29] *[DESCRIBE]*

[27] 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*.

[28] 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.

[29] 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.

Extraction Solvent:

☐ 0.9% Sodium Chloride (SC)

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

☐ Other:[30] *[DESCRIBE]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

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

☐ Other:[31] *[DESCRIBE]*

Extraction Conditions:

☐ 37°C, 72 h

☐ 50°C, 72 h

☐ 70°C, 24 h

☐ 121°C, 1 h

☐ Other:[32] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[33]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[34]

☐ Complete fill with agitation

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

[30] 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.

[31] *Ibid*

[32] *Ibid*

[33] *Ibid*

[34] 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.

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

Extract Observations:
☐ The test article and extract DID NOT change color, and the extract DID NOT appear turbid or have particles.
☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[35]

ASCA Test Method SOP #: *[ASCAIntracut(date/version)]*
☐ Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or
☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[36]

Description of deviations/amendments

Results:[37]

[35] 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.
[36] 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.
[37] 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.

	Test Article	24 h Results	48 h Results	72 h Results	Conclusions
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]

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

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

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

☐ The following adverse clinical findings or animal deaths occurred:[38]

Description of adverse clinical findings or animal deaths

I confirm that:

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

[38] 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.

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

Name: [TYPED NAME POSITION] Date

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

i. Cell line[39] maintenance (e.g., cell line subculture, cell line storage, storage conditions, cell line recovery from storage, use of mycoplasma-free cell line, good cell culture practices, morphology assessment),

ii. Cell counting,

iii. Cell seeding,

iv. Addition of test and control samples to the cell cultures,

v. Scoring of test and control articles including assessment of cellular characteristics (e.g., general cell morphology, vacuolization, detachment, membrane integrity) and percent lysis,

vi. Evaluation criteria and basis for retest,

vii. Data documentation, calculations, analysis and result interpretation (including test-specific assessment of borderline results),

viii. Mock study to assess technician competence in test performance, data documentation, and result interpretation (including test-specific assessment of borderline results). A mock study protocol should be provided to include the following:

- test and control articles used,
- test and control article preparation if this task is conducted by the trainee,
- how test samples and controls are blinded to the trainee,
- test procedure,
- how raw data, analysis and result interpretation will be captured by the trainee and reviewed by the trainer, and
- predefined criteria for assessing a trainee's performance in the mock study to allow them to begin independent ASCA testing.

ix. Minimally, biannual periodic technician proficiency check of negative and positive control scoring. The proficiency check procedure should specify the following:

[39]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.

- 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.

x. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

i. each positive control material replicate is ≥ Grade 3,
ii. each negative control material replicate is Grade 0,
iii. each vehicle control replicate is Grade 0.

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

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*[40]

☐ 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[41] (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]*

_____

[40] 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*.

[41] 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.

☐ Other:[42] *[DESCRIBE]*

Extraction Solvent:

☐ MEM with 5-10% animal serum

☐ Other:[43] *[DESCRIBE]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

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

☐ Other:[44] *[DESCRIBE]*

Extraction Conditions:

☐ 37°C, 24 h

☐ 37°C, 72 h

☐ Other:[45] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[46]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[47]

☐ Complete fill with agitation

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

[42] 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.

[43] 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.

[44] *Ibid*

[45] *Ibid*

[46] *Ibid*

[47] 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.

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

Extract Observations:
☐ The test article and extract DID NOT change color, and the extract DID NOT appear turbid or have particles.
☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[48]

ASCA Test Method SOP #: *[ASCACytotox(date/version)]*
☐ Test was conducted per the above protocol (no deviations/amendments) and 21 CFR 58; or
☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[49]

Description of deviations/amendments

Results:[50]

	24 hour (h) Results (optional)	**48 h Results**	**72 h Results (>48 h device use)**	**Conclusion**
Vehicle Control	Grade 0/0/0	Grade 0/0/0	Grade 0/0/0	Performed as expected
Negative Control: [*Specify per SOP*]	Grade 0/0/0	Grade 0/0/0	Grade 0/0/0	Performed as expected
Positive Control: [*Specify per SOP*]	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
[INSERT ROWS FOR ANY ADDITIONAL TEST ARTICLE DILUTION/RETEST DATA]				

[48] 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.
[49] 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.
[50] 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.

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

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

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

i. Shaving techniques (e.g., to avoid razor burn),
ii. Application of test samples,
iii. Representative sample selection (direct contact),
iv. Differentiation for source of redness (e.g., true irritation versus possible irritation from shaving),
v. Clinical observations (e.g., cage side observation, skin site observation, and presence of adverse events), criteria for assessment, data capture, and frequency (e.g., minimum daily),
vi. Evaluation criteria and basis for retest,
vii. Data documentation, calculations, analysis and result interpretation,
viii. Minimally, biannual periodic technician proficiency check of positive response scoring (in live animals at least once annually). The proficiency check procedure should specify, at a minimum, the following:

- positive and negative controls used,
- number of positive and negative controls, number of animals and/or number of images used (if images are used for proficiency check),
- protocol used to conduct the study, and
- pass/fail criteria. For example, comparison of technician and trainer scores and criteria for acceptable level of agreement: for each individual site, and the overall score (e.g., irritant vs. non-irritant).

ix. Criteria for technician retraining, if needed.

ISO/IEC 17025 Subclause 7.7(a)

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

i. each sodium chloride and oil control site is Grade 0,
ii. confirmation of assay sensitivity by running positive control study at least every six months or tracking that at least one test article positive result has occurred within the previous six months.[51]

[51] ISO 10993-23 ISO 10993-23 Biological evaluation of medical devices – Part 23: Tests for skin irritation.

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

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*[52]

☐ 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[53] (e.g., filtering, extract manipulation, pH adjustment):

Description of deviations/amendments

Test Article Type:

☐ Powder

☐ Solid sample

☐ Test article extracts

[52] 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*.

[53] 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.

☐ Other[54]: [*DESCRIBE*]

Test Article:

☐Entire final finished device

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

☐ Other:[55] *[DESCRIBE]*

Test Article Extraction (if applicable):

Extraction Solvent:

- ☐ 0.9% Sodium Chloride (SC)
- ☐ Cotton Seed Oil (CSO)/Sesame Oil (SO)
- ☐ Other:[56] *[DESCRIBE]*

Extraction Ratio:

- ☐ 6 cm^2/ml (<0.5 mm thick)*
- ☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*
- ☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*
- ☐ 0.2 g/ml (for powder devices)
- ☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

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

- ☐ Other:[57] *[DESCRIBE]*

Extraction Conditions:

- ☐ 37°C, 72 h
- ☐ 50°C, 72 h
- ☐ 70°C, 24 h
- ☐ 121°C, 1 h
- ☐ Other:[58] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

[54] 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.
[55] *Ibid*
[56] *Ibid*
[57] *Ibid*
[58] *Ibid*

☐ Extraction under static conditions or intermittent agitation[59]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[60]

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

Extract Observations:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[61]

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[62]

Description of deviations/amendments

Exposure Time[63]

- ☐ 4 h
- ☐ 4-24 h, specify exposure time:_____
- ☐ Other:[64] *[DESCRIBE]*

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[59] *Ibid*

[60] 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.

[61] 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.

[62] 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.

[63] 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.

[64] 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.

Test Article Application:

☐ 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*]

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

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

☐ Other:[65] *[DESCRIBE]*

Control Article Application:

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

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

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

☐ Other:[66] *[DESCRIBE]*

Results:[67]

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

[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]

[65] 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.

[66] *Ibid*

[67] 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.

For extract-based tests: animal data[68] for both polar and nonpolar test extracts and corresponding vehicle controls should be reported.

^ER = erythema grade; ED = edema grade

Table 2 Summary of Primary Irritation Index*

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

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

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

☐ The following adverse clinical findings or animal deaths occurred:[69]

Description of adverse clinical findings or animal deaths

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

[68] 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

[69] 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.

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

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

xii. Evaluation criteria and basis for pretest, retesting (e.g., invalid control results) and rechallenge,[70] if needed,

xiii. Data documentation, calculations, analysis, and result interpretation (including test-specific assessment of borderline score),

xiv. Minimally, quarterly periodic technician proficiency check of positive control scoring (in live animals at least once annually). The proficiency check procedure should address, at a minimum, the following:

- positive and negative controls used,
- number of animals used (positive control and negative control) and or number of images used (if images are used for proficiency check),
- protocol used to conduct the study,
- evaluation time point(s) used (e.g., 24 hour or 48 hour timepoint or both), and
- pass/fail criteria. For example, comparison of technician and trainer scores and criteria for acceptable level of agreement: for each individual site, and the overall score (e.g., sensitizer vs. non-sensitizer).

xv. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

i. all sodium chloride and oil vehicle control animals have Grade 0 results at all sites for all time points,

ii. the positive controls are run at least biannually (for each animal source and within 3 months of test article test date) and each animal is at least one grade higher than concurrently run sodium chloride and oil vehicle controls in at least 8 out of 10 positive control animals (for strong sensitizers[71] such as 0.1-0.5% dinitrochlorobenzene (DNCB) at induction and 0.05-0.1% DNCB at challenge).

[70] 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.

[71] 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.

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*[72]

☐ 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[73] (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]*

_____

[72] 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*.

[73] 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.

☐ Other:[74] *[DESCRIBE]*

Extraction Solvent:

☐ 0.9% Sodium Chloride (SC)

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

☐ Other:[75] *[DESCRIBE]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

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

☐ Other:[76] *[DESCRIBE]*

Extraction Conditions:

☐ 37°C, 72 h

☐ 50°C, 72 h

☐ 70°C, 24 h

☐ 121°C, 1 h

☐ Other:[77] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[78]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[79]

[74] 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.

[75] 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.

[76] *Ibid*

[77] *Ibid*

[78] *Ibid*

[79] 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.

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

Extract Observations:

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

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[81]

Description of deviations/amendments

Results:[82]

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		

[80] 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.
[81] 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.
[82] 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).

	10	0	0	0	0		
SC Control	**1**	0	0	0	0	0%	Performed as expected
	2	0	0	0	0		
	3	0	0	0	0		
	4	0	0	0	0		
	5	0	0	0	0		
SO 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		
	10	0	0	0	0		
SO Control	**1**	0	0	0	0	0%	Performed as expected
	2	0	0	0	0		
	3	0	0	0	0		
	4	0	0	0	0		
	5	0	0	0	0		
Positive Control* *[Specify]*	**1**	0	2	0	2	100%	Performed as expected
	2	0	2	0	1		
	3	0	2	0	3		
	4	0	2	0	2		
	5	0	2	0	2		

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

***Periodic/concurrent positive control study**

☐ 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]*

☐ The same source, strain, and treatment methods used for positive control testing and done within 3 months of (i.e., before or after) test article test date

☐ The following differences in source, strain, treatment methods or timing of the positive control occurred:[83]

[83] 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.

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

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

☐ The following adverse clinical findings or animal deaths occurred: [84]

Description of adverse clinical findings or animal deaths.

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

[84] *Ibid*

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

i. Shaving techniques (e.g., to avoid razor burn),
ii. Representative sample selection (for direct contact test),
iii. Sample application to test site,
iv. Animal wrapping techniques to prevent restriction of animal breathing or trauma to the site while maintaining test article exposure,
v. Procedure if contact between test or control articles (or patches) and skin is interrupted during exposure (e.g., wrap loosens or fall off),
vi. Differentiation of source of redness during scoring (e.g., true sensitization versus mechanical/adhesive irritation),
vii. Minimization of bias during scoring (e.g., without knowledge of the treatment),
viii. Clinical observations (e.g., cage side observation, skin site observation, and presence of adverse events), criteria for assessment, data capture, and frequency (e.g., minimum daily),
ix. Evaluation criteria and basis for pretest, retesting (e.g., invalid control results) and rechallenge[85], if needed,
x. Data documentation, calculations, analysis and result interpretation (including test-specific assessment of borderline score),
xi. Minimally, quarterly periodic technician proficiency check of positive control scoring (in live animals at least once annually). The proficiency check procedure should address, at a minimum, the following:

- positive and negative controls used,
- number of animals used (positive control and negative control) and or number of images used (if images are used for proficiency check),
- protocol used to conduct the study,

[85] 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.

- evaluation time point(s) used (e.g., 24 hour or 48 hour timepoint or both), and
- pass/fail criteria. For example, comparison of technician and trainer scores and criteria for acceptable level of agreement: for each individual site, and the overall score (e.g., sensitizer vs. non-sensitizer).

xii. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

i. all negative control animals (e.g., sodium chloride or oil vehicles or negative control materials) have Grade 0 results at all sites for all time points,

ii. the positive controls are run at least biannually (for each animal source and within 3 months of test article test date) and each animal is at least one Grade higher than concurrently run sodium chloride and oil vehicle controls in at least 8 out of 10 positive control animals (for strong sensitizers[86] such as 0.1-0.5% DNCB at induction and 0.05-0.1% DNCB at challenge).

B. Example ASCA Summary Test Report: Closed Patch 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*[87]

[86] 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.

[87] 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*.

☐ 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[88] (e.g., filtering, extract manipulation, pH adjustment):

Description of deviations/amendments

Test Article Type:

☐ Powder

☐ Solid sample

☐ Test article extracts

☐ Other:[89] *[DESCRIBE]*

Test Article:

☐ Entire final finished device

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

☐ Other:[90] *[DESCRIBE]*

Test Article Extraction (if applicable):

Extraction Solvent:

☐ 0.9% Sodium Chloride (SC)

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

☐ Other:[91] *[DESCRIBE]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

[88] 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.

[89] 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.

[90] *Ibid*

[91] *Ibid*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*
☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*
☐ 0.2 g/ml (for powder devices)
☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]
*Note: For absorbent device only: [*Specify surface area of test article and the total volume of extraction vehicle used taking into account the additional volume from absorbency determination.*]
☐ Other:[92] *[DESCRIBE]*

Extraction Conditions:

☐ 37°C, 72 h
☐ 50°C, 72 h
☐ 70°C, 24 h
☐ 121°C, 1 h
☐ Other:[93] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation
☐ Extraction under static conditions or intermittent agitation[94]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[95]
☐ Complete fill with agitation
☐ Partial fill with agitation (ISO 10993-12 surface/volume ratio)
☐ Partial fill with agitation (other surface/volume ratio): [*DESCRIBE RATIO USED*]
☐ Other: [*SUMMARIZE APPROACH*]

Extract Observations:

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

[92] 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.
[93] *Ibid*
[94] *Ibid*
[95] 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.

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[96]

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[97]

Description of deviations/amendments

Test Article Application:

☐ 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*]

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

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

☐ Other:[98] *[DESCRIBE]*

Control Article Application:

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

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

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

☐ Other:[99] *[DESCRIBE]*

Results:[100]

[96] 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.

[97] 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.

[98] 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.

[99] *Ibid*

[100] 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.

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		
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		
	10	0	0	0	0		
Negative Control: [*Specify*]	**1**	0	0	0	0	0%	Performed as expected
	2	0	0	0	0		
	3	0	0	0	0		
	4	0	0	0	0		
	5	0	0	0	0		
Concurrent Positive Control: Positive Control ** **[*Specify*]**	**1**	0	2	0	2	100%	Performed as expected
	2	0	2	0	1		
	3	0	2	0	3		
	4	0	2	0	2		
	5	0	2	0	2		

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

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

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

☐ 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]*

☐ The same source, strain, and treatment methods used for positive control testing and done within 3 months of (i.e., before or after) test article test date

☐ The following differences in source, strain, treatment methods or timing of the positive control occurred: [101]

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

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

☐ The following adverse clinical findings or animal deaths occurred:[102]

Description of adverse clinical findings or animal deaths

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

[101] 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.

[102] *Ibid*

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

i. Balance use and calibration to ensure appropriate sensitivity,
ii. Weight range of animals,
iii. Temperature for test sample (e.g., room or body temperature) to be delivered to the animals,
iv. Intraperitoneal (IP) and intravenous (IV) injection techniques and signs to confirm appropriate injection location,
v. Injection rate (e.g., not to exceed 2 ml/min) and how the specified injection rate is achieved,
vi. Evaluation criteria and basis for retest,
vii. Data documentation, calculations, analysis and result interpretation,
viii. Clinical observations by performing a cage-side observation for overt clinical signs using common laboratory descriptors of clinical effects per ASTM F750 and for any mortality along with recording of data, and timing of observations
ix. Minimally, technician proficiency check on injection techniques prior to conduct of next test if it has been more than one month between technician conduct of a study. The proficiency check procedure should specify the following:

- restraining technique,
- injection solutions used (for IV and IP injections),
- number of animals used for proficiency check on injection (for IV and IP), and
- pass/fail criteria. For example, acceptable level of successful injection. Acceptance criteria for successful injection needs to be clearly defined and including details on:

    - ensuring injection rate 2mL/min is achieved,
    - ensuring complete volume of liquid is injected,
    - method to ensure that the needle is in the vein for IV injection, and

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

x. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

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

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

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*[103]

☐ 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

[103] 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*.

☐ Test Article was prepared per the above protocol, with the following deviations/amendments[104] (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]*

☐ Other:[105] *[DESCRIBE]*

Extraction Solvent:

- ☐ 0.9% Sodium Chloride (SC)
- ☐ Cotton Seed Oil (CSO)/Sesame Oil (SO)
- ☐ Other: [106] *[DESCRIBE]*

Extraction Ratio:

- ☐ 6 cm^2/ml (<0.5 mm thick)*
- ☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*
- ☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*
- ☐ 0.2 g/ml (for powder devices)
- ☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

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

- ☐ Other:[107] *[DESCRIBE]*

Extraction Conditions:

- ☐ 37°C, 72 h
- ☐ 50°C, 72 h
- ☐ 70°C, 24 h
- ☐ 121°C, 1 h
- ☐ Other:[108] *[DESCRIBE]*

[104] 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.

[105] 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.

[106] *Ibid*

[107] *Ibid*

[108] *Ibid*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[109]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[110]

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

Extract Observations:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[111]

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[112]

Description of deviations/amendments

Results:[113]

[109] 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.

[110] 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.

[111] 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.

[112] 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.

[113] 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.

Table 1 Summary of Test Results[114]

Extract	Animal Number	Body Weight (g)				Weight Change	Conclusion (Based on Body Weight and Clinical Findings)
		Day 0	Day 1	Day 2	Day 3		
SC Test	**1**	20.4	20.8	21.1	21.7	1.3	No acute systemic toxicity
	2	19.6	20.4	20.3	21.7	2.1	No acute systemic toxicity
	3	19.6	19.9	20.1	20.7	1.1	No acute systemic toxicity
	4	20.4	19.8	20.3	21.1	0.7	No acute systemic toxicity
	5	17.9	18.6	19.0	19.7	1.8	No acute systemic toxicity
SC Control	**1**	17.9	19.9	19.8	20.4	2.5	Performed as expected
	2	19.8	20.0	20.9	22.3	2.5	Performed as expected
	3	19.9	20.3	20.8	21.4	1.5	Performed as expected
	4	17.9	17.8	17.9	18.6	0.7	Performed as expected
	5	22.1	22.9	23.1	24.3	2.2	Performed as expected
SO Test	**1**	22.2	22.9	22.8	23.4	1.2	Not systemically toxic
	2	20.2	21.3	21.4	21.8	1.6	Not systemically toxic
	3	19.0	19.2	19.3	20.2	1.2	Not systemically toxic
	4	18.5	19.8	20.5	21.6	3.1	Not systemically toxic
	5	19.4	202.2	19.8	20.0	0.6	Not systemically toxic
SO Control	**1**	19.7	20.2	20.5	21.9	2.2	Not systemically toxic
	2	19.4	19.9	19.7	20.0	0.6	Not systemically toxic
	3	21.2	21.7	22.2	23.6	2.4	Not systemically toxic
	4	20.9	21.7	22.0	23.1	2.2	Not systemically toxic
	5	20.3	21.1	21.6	23.4	3.1	Not systemically toxic

[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]

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

☐ The following adverse clinical findings or animal deaths occurred:[115]

Description of adverse clinical findings or animal deaths

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.

[114] This is an example of how data from an acute systemic toxicity test could be presented.

[115] 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.

Name: [TYPED NAME POSITION] Date

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

i. Use of "pyrogen-free" /depyrogenated glassware and "pyrogen-free"[116] saline (e.g., USP Sodium Chloride for Injection) for extraction,
ii. Temperature probe use and calibration to ensure appropriate sensitivity,
iii. Noise level maintenance to ensure animals are housed in a quiet environment for testing,
iv. Sham test procedure,
v. Animal restraining and transfer,
vi. Placement of temperature-sensing probe (depth and duration),
vii. Animal temperature monitoring techniques (e.g., continuous monitoring with instrumentation),
viii. Temperature for test sample (i.e., body temperature) to be delivered to the animals,
ix. Intravenous (IV) injection techniques and signs to confirm appropriate injection location (e.g., marginal ear vein) including immediate outcomes, descriptions of observations to ensure that injection was successfully administered within 10 minutes,
x. Evaluation criteria and basis for retest,
xi. Data documentation, calculations, analysis and result interpretation,
xii. Criteria for reusing rabbits across studies (e.g., between different studies and over an animal's life), and
xiii. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

[116] 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

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

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*[117]

☐ 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[118] (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]*

[117] 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*.

[118] 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.

☐ Other:[119] *[DESCRIBE]*

Extraction Solvent:

☐ 0.9% Sterile "Pyrogen-Free"[120] Saline

☐ Other:[121] *[DESCRIBE]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

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

☐ Other:[122] *[DESCRIBE]*

Extraction Conditions:

☐ 37°C, 72 h

☐ 50°C, 72 h

☐ 70°C, 24 h

☐ 121°C, 1 h

☐ Other:[123] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[124]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

[119] 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.

[120] 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

[121] 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.

[122] *Ibid*

[123] *Ibid*

[124] *Ibid*

Fluid Path Extractions:

☐ 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:[125]

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

Extract Observations:

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

☐ There were changes in color/ turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[126]

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[127]

Description of deviations/amendments

Results:[128]

Table 1 Pyrogen Test Data[129]

[125] 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.

[126] 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.

[127] 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.

[128] The complete test report should be included with ASCA Summary Test Report if any rabbit has a baseline temperature exceeding 39.8ºC or if any rabbit has a temperature rise ≥0.5°C.

[129] This is an example of how data from a material-mediated pyrogenicity test could be presented.

Animal Number	Baseline Temp (°C)	1.0 hour (h) Temp (°C)	1.5 h Temp (°C)	2.0 h Temp (°C)	2.5 h Temp (°C)	3.0 h Temp (°C)	Temp Increase (°C)	Conclusion
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			

[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]

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

☐ The following adverse clinical findings or animal deaths occurred:[130]

Description of adverse clinical findings or animal deaths

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

[130] 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.

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

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

- test and control articles used,
- test procedure (including recognition and correction of background interference in extracts or supernatants),

- how raw and corrected data, documentation of the procedure, analysis, and interpretation of results will be captured by the trainee and reviewed by the trainer,
- predefined criteria for assessing a trainee's performance in the mock study to allow them to begin independent ASCA testing.

xviii. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

i. the positive control material mean hemolytic index is ≥ 5%,
ii. the negative control material mean hemolytic index is < 2%,
iii. the negative control material "Blank Corrected % Hemolysis" value for any replicate is ≥ -1%.

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

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*[131]

☐ 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)]*

[131] 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*.

☐ 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[132] (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]*
☐ Other:[133] *[DESCRIBE]*

Extract testing

Extraction Solvent:
☐ Magnesium and Calcium Free PBS
☐ Other:[134] *[DESCRIBE]*

Extraction Ratio:
☐ 6 cm^2/ml (<0.5 mm thick)*
☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0mm)*
☐ 1.25 cm^2/ml (elastomers > 1.0mm thick)*
☐ 0.2 g/ml (for powder devices)
☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]
*Note: For absorbent device only: [*Specify surface area of test article and the total volume of extraction vehicle used taking into account the additional volume from absorbency determination.*]
☐ Other:[135] *[DESCRIBE]*

Extraction Conditions:
☐ 37°C, 72 h
☐ 50°C, 72 h
☐ 70°C, 24 h
☐ 121°C, 1 h

[132] 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.
[133] 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
[134] *Ibid*
[135] *Ibid*

☐ Other:[136] *[DESCRIBE]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[137]: *[DESCRIBE and PROVIDE JUSTIFICATION]*

Fluid Path Extractions:

☐ 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:[138]

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

Extract Observations (Post-extraction):

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[139]

Post-incubation and centrifugation of blood solutions:

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

☐ The test article red blood cell pellet after centrifugation WAS visually different in color or size (e.g., larger or very small) compared to the pellet for the negative control. [140]

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

☐ There were changes in color/turbidity or particles in the test article supernatant.[141]

[136] 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.
[137] 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.
[138] 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.
[139] 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.
[140] *Ibid*
[141] *Ibid*

Direct Contact

Diluent:

☐ Magnesium and Calcium Free PBS

☐ Other:[142] *[DESCRIBE]*

Exposure Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)

☐ 0.2 g/ml (for powder devices)

☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

☐ Other:[143] *[DESCRIBE]*

Post-incubation and centrifugation of blood solutions:

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

☐ The test article red blood cell pellet after centrifugation WAS visually different in color or size (e.g., larger or very small) compared to the pellet for the negative control. [144]

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

☐ There were changes in color/turbidity or particles in the supernatant OR there was swelling/degradation of the test article.[145]

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments: [146]

Description of deviations/amendments

[142] 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.

[143] 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.

[144] *Ibid*

[145] *Ibid*

[146] 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.

Results:[147]

[147] 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 ≥2% .

2177 **Table 1 Hemolysis test results[148]**

Extract Hemolysis									
Sample	**Absorbance**			**Blank corrected % hemolysis**			**Mean Blank Corrected Hemolysis (%)**	**Hemolytic Index (%)**	**Conclusions**
	Replicate #1	**Replicate #2**	**Replicate #3**	**Replicate #1**	**Replicate #2**	**Replicate #3**			
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
Direct Hemolysis									
Sample	**Absorbance**			**Blank corrected % hemolysis**			**Mean Blank Corrected Hemolysis (%)**	**Hemolytic Index (%)**	**Conclusions**
	Replicate #1	**Replicate #2**	**Replicate #3**	**Replicate #1**	**Replicate #2**	**Replicate #3**			

[148] This is an example of how data from a hemolysis test could be presented.

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]

Calibration Coefficient (F): _____

Diluted Blood Hemoglobin Concentration (mg/ml)[149]: _____

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

[149]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.

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

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

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

xiv. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

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

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

- the mean value for the cobra venom factor positive control (if applicable) is at least 10X greater than both the mean values for the negative control material and the activated normal human serum, plasma, or whole blood, or
- the positive material control (if applicable) is statistically significantly higher than both the negative control material and the activated normal human serum, plasma, or whole blood.

ii. any kit-specific high and low controls meet the kit specifications,

iii. SC5b-9 complement activation assay validation study protocol and report[150] provided to demonstrate that the test method can clearly differentiate materials with different complement activation potentials (i.e., low, moderate, and high).

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

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*[151]

☐ 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.

[150] 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.

[151] 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*.

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[152] (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]*

☐ Other: [153]*[DESCRIBE]*

US Marketed Comparator Device[154] (optional):

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

☐ Entire final finished device

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

☐ Other: [155]*[DESCRIBE]*

Test Medium:

☐ Normal Human Serum

☐ Human Plasma

☐ Whole Blood

☐ Other:[156]*[DESCRIBE]*

Exposure Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm)

☐ 0.2 g/ml (for powder devices)

[152] 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.

[153] 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.

[154] 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.

[155] 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.

[156] *Ibid*

☐ 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) [*Provide information on comparison when mass/volume ratio versus surface area/volume ratio is used.*]

☐ Other:[157] *[DESCRIBE]*

Exposure Conditions:

☐ 37°C for 60-90 min

☐ Other:[158] *[DESCRIBE]*

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

☐ There were changes in color/turbidity or particles in the supernatant OR there was swelling/degradation of the test article.[159]

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[160]

Description of deviations/amendments

Manufacturer of SC5b-9 ELISA kit: _____

Results:[161]

[157] 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.

[158] 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.

[159] *Ibid*

[160] 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.

[161] 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.

Table 1 SC5b-9 Protein Concentration (ng/mL)[162]

Samples	Dilution	Concentration (ng/mL)					
		Replicate #1	Replicate #2	Replicate #3	Mean	Std	Conclusion
Test Article	1:160	208	216	212	212	3.8	Not a complement activator*
Test Medium Control [*Specify (e.g., blood, serum, or plasma)*]	1:160	205	207	208	207	1.5	Performed as expected
Negative Control Material: [*Specify*]	1:160	206	205	204	205	1.1	Performed as expected
Positive Control Material:[163][*Specify*]	1:160	683	693	688	688	4.8	Performed as expected
Cobra Venom Factor Positive Control[164]	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

[INSERT ROWS FOR ANY ADDITIONAL RETEST DATA]

*not statistically different from negative or comparator controls

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

[162] This is an example of how data from a complement activation test could be presented.
[163] Depending on the positive control used, this row may be relevant.
[164] *Ibid*

Appendix L: Example ASCA Test Specific Controls

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

ASCA Test Method	Control/Reagent
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)

	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

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

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

ISO/IEC 17025 Subclause 7.7(a)

The testing laboratory agrees that pre-defined criteria for positive/negative/reference control values will be as follows[166]:

[165] 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.

[166] See ISO 10993-5 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.

iv. The mean OD_{570} of media control (i.e., media with cells) is ≥0.2,
v. The means of the "Left Media Control" and "Right Media Control" do not differ by more than 15% of the mean of all media control replicates,
vi. The viability of each positive control material (e.g., ZDEC or ZDBC[167]) replicate is less than 70% of the mean of all media control replicates,
vii. The viability of each negative control replicate is greater than 70% of the mean of all media control replicates, and
viii. The viability of 50% extract dose of each positive control material (e.g., ZDEC or ZDBC) replicate has at least the same or higher viability than the 100% extract dose of the positive control.

[167] ZDEC and ZDBC are organotin-stabilized polyurethanes.

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

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*[168]

☐ 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[169] (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]*

☐ Other:[170] *[Describe]*

[168] 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*.

[169] 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.

[170] 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.

Extraction Solvent:

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

☐ Other:[171] *[Describe]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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)

*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]*

☐ Other:[172] *[Describe]*

Extraction Conditions:

☐ 37°C, 24 h

☐ 37°C, 72 h

☐ Other:[173] *[Describe]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[174]: *[Describe and provide justification]*

Fluid Path Extractions:

☐ 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: [175]

☐ Complete fill with agitation

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

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

☐ Other: *[Summarize approach]*

[171] 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.

[172] *Ibid*

[173] *Ibid*

[174] *Ibid*

[175] 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.

Test Article and Extract Appearance:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[176]

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.

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[177]

Description of deviations/amendments

[176] 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.

[177] 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.

Results:

Table 1: Optical Density (OD) and Cell Viability Data [178]

	Optical Density (*OD_{570})					†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)						

	Optical Density (OD_{570})				
	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

*OD_{570}: optical density reading with a 570 nm filter with a reference wavelength of 650 nm.

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

^SD: Standard Deviation

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

‡ HDPE: high-density polyethylene

[178] 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.

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)			

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

I confirm that the following test validity criteria[179] are met:

☐ The mean OD_{570} of the media control (i.e., media with cells) is ≥0.2.
☐ The means of the "Left Media Control" and "Right Media Control" do not differ by more than 15% of the mean of all media control replicates.
☐ The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than 70% of the mean of all media control replicates.
☐ The viability of each negative control replicate is greater than 70% of the mean of all media control replicates.
☐ The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC) replicate has at least the same or higher viability than the 100% extract dose of the positive control.
☐ The viability of the 50% extract dose of each test article replicate has at least the same or higher viability than the 100% extract dose of the test article.

Overall Results:

☐ Cytotoxic[180]. Any individual replicate of the test article extract (100%) or dilution of the test article extract resulted in a % viability lower than 70%.
☐ Non-cytotoxic. All individual replicate of the test article extract (100%) or dilution of the test article extract resulted in a % viability 70% or greater.

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

[179] 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.
[180] 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.

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

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

ISO/IEC 17025 Subclause 6.2(e)

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

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

[181] 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.

xix. Criteria for technician retraining.

ISO/IEC 17025 Subclause 7.7(a)

The testing laboratory agrees that pre-defined criteria for positive/negative/reference control values will be as follows[182]:

i. The mean OD_{540} of the media controls (i.e., media with cells) is ≥ 0.3,
ii. The means of the "Left Media Control" and "Right Media Control" do not differ by more than 15% of the mean of all media control replicates,
iii. The IC_{50} for Sodium Lauryl Sulfate (SLS) falls within the 95% confidence interval of 0.093 mg/mL (i.e., between 0.07 mg/mL to 0.116 mg/mL),
iv. The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than 70% of the mean of all media control replicates,
v. The viability of each negative control replicate is greater than 70% of the mean of all media control replicates, and
vi. The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC) replicate has at least the same or higher viability than the 100% extract dose of the positive control.

[182] See ISO 10993-5 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.

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

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*[183]

☐ 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[184] (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]*

☐ Other:[185] *[Describe]*

[183] 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*.

[184] 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.

[185] 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.

Extraction Solvent:

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

☐ Other:[186] *[Describe]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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)

*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*]

☐ Other:[187] *[Describe]*

Extraction Conditions:

☐ 37°C, 24 h

☐ 37°C, 72 h

☐ Other:[188] *[Describe]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[189]: *[Describe and provide justification]*

Fluid Path Extractions:

☐ 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:[190]

- ☐ Complete fill with agitation
- ☐ Partial fill with agitation (ISO 10993-12 surface/volume ratio)
- ☐ Partial fill with agitation (other surface/volume ratio): *[Describe ratio used]*
- ☐ Other: *[Summarize approach]*

[186] 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.

[187] *Ibid*

[188] *Ibid*

[189] *Ibid*

[190] 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.

Test Article and Extract Appearance:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[191]

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.

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[192]

Description of deviations/amendments

[191] 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.

[192] 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.

Results:

Table 1: Optical Density and Cell Viability Data[193]

	Optical Density (*OD_{540})					†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

	Optical Density (OD_{540})				
	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					

*OD_{540}: optical density reading with a 540 nm filter using the blanks as a reference.

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

^SD: Standard Deviation

†Viability%=$\frac{mean\ value\ of\ OD_{540}\ of\ the\ test\ articl\ extract}{mean\ value\ of\ \ OD_{540}\ of\ the\ medium\ control} \times 100\%$

‡ HDPE: high-density polyethylene

[193] 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.

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%)			
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)			

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

I confirm that the following test validity criteria[194] are met:

☐ The mean OD_{540} of the media control (i.e., media with cells) is ≥0.3.
☐ The means of the "Left Media Control" and "Right Media Control" do not differ by more than 15% of the mean of all media control replicates.
☐ The IC 50 for SLS falls within the 95% confidence interval of 0.093 mg/ml (i.e., between 0.07 mg/mL to 0.116 mg/mL).
☐ The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than 70% of the mean of all media control replicates.
☐ The viability of each negative control replicate is greater than 70% of the mean of all media control replicates.
☐ The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC) replicate has at least the same or higher viability than the 100% extract dose of the positive control.
☐ The viability of the 50% extract dose of each test article replicate has at least the same or higher viability than the 100% extract dose of the test article.

Overall Results:

☐ Cytotoxic[195]. Any individual replicate of the test article extract (100%) or dilution of the test article extract resulted in a % viability lower than 70%.

☐ Non-cytotoxic. All individual replicate of the test article extract (100%) or dilution of the test article extract resulted in a % viability 70% or greater.

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

[194] 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.
[195] 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.

Appendix O: Test Method-Specific ASCA Specifications and Summary Test Report –XTT Cytotoxicity (ISO 10993-5)

A. ASCA Specifications: XTT Cytotoxicity (ISO 10993-5)

ISO/IEC 17025 Subclause 6.2(e)

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

i. Cell line[196] maintenance (e.g., cell line subculture, cell line storage, storage conditions, cell line recovery from storage, use of mycoplasma-free cell line, good cell culture practices, morphology assessment),
ii. Cell counting,
iii. Cell seeding,
iv. Preparation and storage of XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyaniline inner salt) solution and PMS (phenazine methosulfate) solution,
v. Check for cell seeding errors and growth characteristics of control and treated cells,
vi. Multichannel pipetting,
vii. Cell culture medium removal from the 96 well plates,
viii. Addition of test and control samples to the cell cultures,
ix. Preparation of different doses of test extract and positive controls, if needed.
x. Microscopic evaluation of cell morphology,
xi. Addition of XTT/PMS solution mixture to cells and incubation,
xii. Transfer of test, control, and blank samples for absorbance reading,
xiii. Use of microplate reader, calibration, and blank correction,
xiv. Evaluation criteria and basis for retest,
xv. Data documentation, calculations, analysis and result interpretation,
xvi. Mock study to assess technician competence in test performance, data documentation, and result interpretation. A mock study protocol should be provided to include, at a minimum, the following:
   - test and control articles used,
   - test and control article preparation if this task is conducted by the trainee,
   - how test samples and controls are blinded to the trainee,
   - test procedure,
   - how raw data, analysis and result interpretation will be captured by the trainee and reviewed by the trainer, and
   - predefined criteria for assessing a trainee's performance in the mock study to allow them to begin independent ASCA testing.
xvii. Criteria for technician retraining.

[196]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.

ISO/IEC 17025 Subclause 7.7(a)

The testing laboratory agrees that pre-defined criteria for positive/negative/reference control values will be as follows[197]:

i. The mean OD_{450} of the media control (i.e., media with cells) is ≥ 0.2,
ii. The means of the "Left Media Control" and "Right Media Control" do not differ by more than 15% of the mean of all media control replicates,
iii. The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than 70% of the mean of all media control replicates,
iv. The viability of each negative control replicate is greater than 70% of the mean of all media control replicates, and
v. The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC) replicate has at least the same or higher viability than the 100% extract dose of the positive control.

[197] See ISO 10993-5 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity.

B. Example ASCA Summary Test Report: XTT Cytotoxicity (ISO 10993-5)

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*[198]
   - ☐ 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[199] (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]*

☐ Other:[200] *[Describe]*

Extraction Solvent:

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

[198] 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*.

[199] 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.

[200] 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.

☐ Other:[201] *[Describe]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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)

*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]*

☐ Other:[202] *[Describe]*

Extraction Conditions:

☐ 37°C, 24 h

☐ 37°C, 72 h

☐ Other:[203] *[Describe]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[204]: *[Describe and provide justification]*

Fluid Path Extractions:

☐ 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:[205]

☐ Complete fill with agitation

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

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

☐ Other: *[Summarize approach]*

[201] *Ibid*

[202] 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.

[203] *Ibid*

[204] *Ibid*

[205] 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.

Test Article and Extract Appearance:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[206]

to support why changes in the test article and/extract are acceptable.

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

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[207]

Description of deviations/amendments

[206] 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.

[207] 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.

Results:

Table 1: Optical Density and Cell Viability Data[208]

	*Blank Corrected Optical Density (OD_{450})					†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)						

	Optical Density (OD_{450})				
	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

*Blank corrected optical density (OD450): Optical density reading with a 450 nm filter and a reference wavelength of 630 nm using cell culture media without cells for blank correction. Blank is cell culture media without cells.

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

^SD: Standard Deviation

†Viability%=$\frac{mean\ value\ of\ OD_{450}\ of\ the\ test\ articl\ extract}{mean\ value\ of\ \ OD_{450}\ of\ the\ medium\ control} \times 100\%$

‡ HDPE: high-density polyethylene

[208] 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.

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)			

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

I confirm that that the following test validity criteria[209] are met:

☐ The mean OD_{450} of the media control (i.e., media with cells) is ≥0.2.
☐ The means of the "Left Media Control" and "Right Media Control" do not differ by more than 15% of the mean of all media control replicates.
☐ The viability of each positive control material (e.g., ZDEC or ZDBC) replicate is less than 70% of the mean of all media control replicate.
☐ The viability of each negative control replicate is greater than 70% of the mean of all media control replicates.
☐ The viability of the 50% extract dose of each positive control material (e.g., ZDEC or ZDBC) replicate has at least the same or higher viability than the 100% extract dose of the positive control.
☐ The viability of the 50% extract dose of each test article replicate has at least the same or higher viability than the 100% extract dose of the test article.

Overall Results:

☐ Cytotoxic[210]. Any individual replicate of the test article extract (100%) or dilution of the test article extract resulted in a % viability lower than 70%.
☐ Non-cytotoxic. All individual replicates of the test article extract (100%) or dilution of the test article extract resulted in a % viability of 70% or greater.

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

[209] 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.
[210] 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.

Appendix P: Test Method-Specific ASCA Specifications and Summary Test Report –Bacterial Reverse Mutation Test (ISO 10993-3 and OECD 471[211])

A. ASCA Specifications: Bacterial Reverse Mutation Assay (i.e., Ames Assay) (ISO 10993-3 and OECD 471)

ISO/IEC 17025 Subclause 6.2(e)

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

i. Bacterial cell culture techniques including stock culture preparation, and bacterial culture maintenance and storage,
ii. Bacterial strain phenotypic marker verification,
iii. Verification of cell density before testing,
iv. Verification of spontaneous revertant colony plate counts,
v. Verification of plate sterility,
vi. Preparation of medium, test article and test article extracts, and controls (i.e., negative and positive),
vii. Preparation and quality control of S9 fraction,
viii. Plate preparation,
ix. Exposure of bacterial cells to controls and test article extracts (e.g., plate incorporation method, preincubation method),
x. Criteria for use of preincubation method vs. plate incorporation method,
xi. Use and calibration of automated colony counters, if used,
xii. Revertant colony counting,
xiii. Evaluation of bacterial background lawn,
xiv. Interpretation of results,
xv. Mock study to assess technician competence in test performance, data documentation, and result interpretation. A mock study protocol should be provided to include, at a minimum, the following:
    - test article, negative (vehicle) controls, and positive controls used,
    - preparation of test article, negative controls, and positive controls, if this task is conducted by the trainee,
    - how test samples and controls are blinded to the trainee,
    - test procedure,
    - revertant colony counting, colony sizing, and discrimination of small colonies vs. large colonies,
    - how raw data, analysis and result interpretation will be captured by the trainee and reviewed by the trainer, and

[211] 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."

- predefined criteria for assessing a technician's performance in the mock study to allow them to begin independent ASCA testing.

xvi. Criteria for technician retraining.

ISO/IEC 17025 Clause 7.2 c)

7.2 c) The testing laboratory agrees that test procedures will include or specify, as appropriate, the following:

i. Test system (i.e., bacterial strains) used, which should include at least 5 bacterial strains with the following combination of strains:
   a. Salmonella typhimurium TA1535, and
   b. Salmonella typhimurium TA1537 or TA97 or TA97a, and
   c. Salmonella typhimurium TA98, and
   d. Salmonella typhimurium TA100, and
   e. Escherichia coli WP2 uvrA, or Escherichia coli WP2 uvrA (pKM101), or Salmonella typhimurium TA102.

ii. Cell density (e.g., approximately 10^9 cells/ml) and confirmation of cell density prior to testing,

iii. Procedure for phenotypic characterization of tester strains prior to testing,

iv. Procedure for use of plate incorporation method or/and preincubation method,

v. Procedure for establishing spontaneous revertant rate for each tester strain,

vi. Test medium, including a description of medium preparation,

vii. S9 fraction (a cofactor-supplemented post-mitochondrial fraction) type (e.g., rat liver S9 fraction), source of S9 fraction (e.g., in-house prepared or name of commercial supplier and product name), and how S9 fraction is qualified (e.g., Certificate of Analysis of the product used, verification testing) before testing. If prepared in-house, specify the preparation method, rodent strain, enzyme-inducing agents (e.g., Aroclor 1254), concentration of cofactors, purity, and verification criteria for S9 activity. For the S9 mixture (e.g., S9 fraction and cofactors), specify the amount or percentage of S9 fraction and concentration of co-factors in the mixture, and the source of the S9 mixture (e.g., in-house or name of commercial supplier and product name).

viii. Procedure to ensure absence of microbial contamination prior to plating,

ix. Procedure for test article preparation and extract storage,

x. Procedure and criteria for cytotoxicity determination of test extract[212]. The test extract is considered cytotoxic if one or both of the following criteria are met for all tester strains:
   - Greater than 50% reduction in the number of revertant colonies,
   - At least a moderate reduction in the background lawn.

xi. Procedure for exposure of bacterial cells to the test article and controls with and without S9,

xvii. Procedure for use of undiluted extracts unless cytotoxicity is observed, in which case, dilutions of extracts should also be tested,

[212] If cytotoxicity is observed with the test extract, a complete test report should be provided.

xii. Procedure for colony counting,
xiii. Procedure for preparation of positive and negative (vehicle) concurrent controls including final concentrations (e.g., amount of positive control per plate). Negative controls should be handled in a manner similar to the device extract (i.e., incubated under the same conditions).
xiv. Procedure for generation of historical negative (vehicle) and positive control data (e.g., ranges, means and standard deviations), criteria for inclusion and exclusion of historical studies in the historical data cohorts, and how the expected range of revertant colony plate counts is established based on historical negative (vehicle) and positive control data[213]. The historical control data set should initially be built with at least 20 experiments conducted under comparable testing conditions as used for medical device testing[214]. The distribution of the data together with appropriate descriptive statistics should be provided (e.g., confidence intervals, 95-99% percentiles).
xv. Procedure for when repeat test is needed.

ISO/IEC 17025 Subclause 7.7(a)

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

i. The phenotypic characterization of each tester strain demonstrates appropriate genetic markers per OECD 471,
ii. Cell density for each tester strain before testing should be approximately 1 x 10^9 cells ml,
iii. Each concurrent negative (vehicle) control has spontaneous revertant colony plate counts for each tester strain within the frequency ranges of the laboratory's historical control data and within the range reported in the literature,
iv. The mean of the number of revertant colonies in each positive control should exhibit significant increase compared to that in the respective solvent control,
v. Each concurrent positive control material replicate is at least 3 times that of the concurrent negative control for each tester strain[215],
vi. The background lawns of negative (vehicle) controls are normal per ISO/TR 10993-33: 2015[216], Table 2,
vii. Test extract is considered acceptable based on the cytotoxicity evaluation criteria as defined in Section 7.2 c). x. above.

[213]Testing laboratories should have historical data available when applying for ASCA accreditation, and this data should be consistent with the literature.
[214] Hayashi, M, et.al., Compilation and Use of Genetic Toxicity Historical Control Data, Mutation Res., 2011, 723 (2): 87-90.
[215] 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.
[216] ISO/TR 10993-33: 2015 Biological evaluation of medical devices Part 33: Guidance on tests to evaluate genotoxicity Supplement to ISO 10993-3.

B. Example ASCA Summary Test Report: Bacterial Reverse Mutation Assay (i.e., Ames Assay) (ISO 10993-3 and OECD 471)

Note: This example is intended to illustrate the supplemental documentation that would accompany the Declaration of Conformity per FDA's guidance Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices. *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*[217]

☐ 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[218] (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]*

☐ Other:[219] *[Describe]*

[217] 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*.

[218] 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.

[219] 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.

Extraction Solvent:

☐ 0.9% Saline
☐ Dimethyl Sulfoxide (DMSO)
☐ Ethanol *[Provide justification for using ethanol instead of DMSO (e.g., documentation of device degradation in DMSO)]*
☐ Polyethylene glycol 400 (PEG 400) *[Provide justification for using PEG instead of DMSO (e.g., documentation of device degradation in DMSO)]*
☐ Other:[220] *[Describe]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*
☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)*
☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*
☐ 0.2 g/ml (for powder devices)
☐ 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)
*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]*
☐ Other:[221] *[Describe]*

Extraction Conditions:

☐ 37°C, 72 h
☐ 50°C, 72 h
☐ 70°C, 24 h
☐ 121°C, 1 h
☐ Other:[222] *[Describe]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation
☐ Extraction under static conditions or intermittent agitation[223]: *[Describe and provide justification]*

[220] 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.
[221] *Ibid*
[222] *Ibid*
[223] *Ibid*

Fluid Path Extractions:

☐ 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[224]:

- ☐ Complete fill with agitation
- ☐ Partial fill with agitation (ISO 10993-12 surface/volume ratio)
- ☐ Partial fill with agitation (other surface/volume ratio): *[Describe ratio used]*
- ☐ Other: *[Summarize approach]*

Test Article and Extract Appearance:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[225]

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.

ASCA Test Method SOP #: *[#######-ASCAAmes(date/version)]*

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[226]

Description of deviations/amendments

[224] 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.

[225] 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.

[226] 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.

Results

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	☐ TA98						
	☐ TA1535						
	☐ TA100						
	☐ TA97						
	☐ TA97a						
	☐ TA1537						
	☐ TA102						
Escherichia coli	☐ WP2 uvrA						
	☐ WP2 uvrA (pKM101)						

(*Select strains that are used in testing and record phenotypic characterization results for each bacterial strain. If the phenotypic test is not applicable to the strain, the box is greyed out)

I confirm that the bacterial counts for each tester strain before testing were:

☐ Approximately 1 x 10^9 cells/ml[227]

[227] 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.

Table 2: Positive Control [specify positive control] and concentration for each tester strain]

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)**

Table 3: Historical Positive and Negative Control Colony Count Data [specify testing period] *

	Tester Strain	Negative Control Colony Counts			Positive Control Colony Counts		
		Range	Mean ±SD	Number of Data Points	Range	Mean ±SD	Number of Data Points
Without S9							
With S9							

* Historical negative and positive control data should be built with at least 20 experiments (i.e., 20 data points for each tester strain with and without S9 fraction) under comparable testing conditions as used for medical device testing.

3080 **Table 4: Test Article and Concurrent Control Colony Count Data**

		With S9 Activation					Without S9 Activation				
	▫Replicate Number	*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										

*Specify test article polar extract (e.g., test article saline extract), polar vehicle control (e.g., saline), test article non-polar extract (e.g., test article DMSO extract), non-polar vehicle control (e.g., DMSO), and positive control in the results table per SOP. Commonly used polar extraction vehicle is 0.9% saline. Commonly used non-polar extraction vehicle is DMSO, ethanol, and polyethylene glycol 400 (PEG 400)[228].

¤At least 3 replicates should be used for each test article and control.

·SD=Standard Deviation

†FI=Fold increase = $\frac{mean\ test\ article\ colony\ count\ value}{mean\ vehicle\ control\ colony\ count\ value}$

[228] 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.

Table 5: Background Lawn◊ Observation[229]

		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										

* Specify test article polar extract (e.g., test article saline extract), polar vehicle control (e.g., saline), test article non-polar extract (e.g., test article DMSO extract), non-polar vehicle control (e.g., DMSO), and positive control in the results table per SOP.

◊ Describe background lawn using criteria defined in Table 2 of ISO/TR 10993-33: normal (1), slightly reduced (2), moderately reduced (3), extremely reduced (4), etc.

[229] 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.

I confirm that that the following test validity criteria are met[230]:

☐ The phenotypic characterization of each tester strain demonstrates normal results.

☐ Each concurrent negative control has spontaneous revertant colony plate counts for each tester strain within the frequency ranges of the laboratory's historical control data and within the range reported in the literature.

☐ The mean of the number of revertant colonies in each positive control shows significant increase compared with that of the respective solvent control.

☐ Each concurrent positive control material replicate is at least 3 times the concurrent negative control for each tester strain[231].

☐ The background lawns of negative controls are normal.

☐ The test extract is considered acceptable based on the cytotoxicity evaluation criteria as defined in Section 7.2 c. x.) above.

Overall Results:

☐ The test article demonstrated a positive response in one or more tester strains when tested with or without S9 fraction[232]. The results are positive if the mean mutant colony counts are two or more times greater than the respective concurrent negative control mean for the tester strains TA98, TA100, TA 97, TA 102 and the two E. coli strains and three or more times greater than the respective concurrent negative control mean for the tester strains TA1535 and TA1537.

☐ The test article demonstrated a negative response in all tester strains when tested with or without S9 fraction. The results are negative if the mean mutant colony counts are less than two times of the respective concurrent negative control mean for the tester strains TA98, TA100, TA 97, TA 102 and the two E. coli strains and less than three times of the respective concurrent negative control mean for the tester strains TA1535 and TA1537.

☐ The test article demonstrated an equivocal response (i.e., elevated mutant colony counts that don't meet the criteria for a positive response) in one or more tester strains under any test conditions (i.e., with or without S9 fraction)[233].

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.

[230] 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.

[231] 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.

[232] 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

[233] *Ibid*

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

Appendix Q: Test Method-Specific ASCA Specifications and Summary Test Report –Mouse Lymphoma Assay (MLA) (ISO 10993-3 and OECD 490[234])

A. ASCA Specifications: MLA (ISO 10993-3 and OECD 490)

ISO/IEC 17025 Subclause 6.2(e)

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

i. Mouse lymphoma cell line[235] maintenance including checks for: mycoplasma contamination, karyotype stability of the cells, modal number of chromosomes, and doubling time; and cleansing of pre-existing mutant cells prior to use in the assay,
ii. Cell culture media, cloning media, test article, and control (negative and positive) preparation,
iii. Preparation and quality control of S9 fraction,
iv. Treatment of cells with test and control articles,
v. Techniques for cell washing and dilution, determination of viable cell concentration, and adjustment to study-specified cell concentrations,
vi. Cell cloning and determination of cloning efficiency,
vii. Soft agar method (if applicable):
    - Cell and cloning agar media mixing with and without the mutant selective agent (e.g., trifluorothymidine (TFT)),
    - Colony counting (i.e., number of colonies), colony sizing, and criteria for small colonies vs. large colonies
viii. Microwell method (if applicable):
    - Cell plating into 96 well plates with and without the mutant selective agent (e.g., TFT),
    - Colony counting (i.e., number of wells with colonies), and discrimination of small colonies vs. large colonies
ix. Cytotoxicity measurement, including determination of relative total growth,
x. Determination of relative total growth, relative cloning efficiency, induced mutant frequency, small colony mutant frequency,
xi. Evaluation criteria and basis for retest,
xii. Data documentation, calculations, analysis and result interpretation,
xiii. Mock study to assess technician competence in test performance, data documentation, and result interpretation. A mock study protocol should be provided to include, at a minimum, the following:
    - test and control articles used,
    - test and control article preparation if this task is conducted by the trainee,
    - how test samples and controls are blinded to the trainee,

[234] OECD 490 Guidelines for the Testing of Chemicals – In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene.

[235] Only L5178Y TK+/- -3.7.2C mouse lymphoma cell line (generally called L5178Y) is allowed for MLA under ASCA.

- colony counting, colony sizing, and discrimination of small colonies vs. large colonies,
- test procedure,
- how raw data, analysis and result interpretation will be captured by the trainee and reviewed by the trainer,
- predefined criteria for assessing a technician's performance in the mock study to allow them to begin independent ASCA testing.

xiv. Technician retraining, if needed.

ISO/IEC 17025 Clause 7.2 c)

7.2 c) The testing laboratory agrees that test procedures will include or specify, as appropriate, the following:

i. Cell density prior to dosing and during expression period,
ii. Procedure for cell culture maintenance and quality control of mouse lymphoma cells including identity, absence of mycoplasma, sensitivity to positive control, stable spontaneous mutant frequency,
iii. Stock culture preparation, including periodic cleansing of spontaneous mutant cells,
iv. Test medium including a description of medium preparation (e.g., serum concentration, S9 fraction concentration),
v. S9 fraction (a cofactor-supplemented post-mitochondrial fraction) type (e.g., rats liver S9 fraction), source of S9 fraction (e.g., in-house prepared or name of commercial supplier and product name), and how S9 fraction is qualified (e.g., Certificate of Analysis of the product used, verification testing) before testing. If prepared in-house, specify the preparation method, rodent strain, enzyme-inducing agents (e.g., Aroclor 1254), concentration of cofactors, purity, and verification criteria for S9 activity. For the S9 mixture (e.g., S9 fraction and cofactors), specify the amount or percentage of S9 fraction and co-factors in the mixture, and the source of the S9 mixture (e.g., in-house or name of commercial supplier and product name).
vi. Procedure for test article extract preparation and extract storage,
vii. Procedure for test article extract dose preparation. For example, if RPMI medium is used for extraction, the undiluted extract supplemented with serum should be used for testing. If 0.9% saline is used for extraction, dilution to 10% (v/v) in RPMI medium with serum should be used for testing. If non-polar solvents are used for extraction, dilution to 1% (v/v) in RPMI medium with serum should be used for testing. For mouse lymphoma assay, commonly used non-polar solvents for extraction are DMSO, ethanol, PEG 400[236].
viii. Procedure for concurrent control (positive, negative) preparations, including final concentrations. Negative controls should be handled in a manner similar to the device extract (i.e., incubated under the same conditions).

[236] 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.

ix. Procedure for exposure of cells to test articles and controls (4-hour treatment with and without S9, and 24-hour treatment without S9),
x. Procedure and criteria for cytotoxicity determination of test extract and positive controls (i.e., relative total growth),
xi. Procedure for mutant expression and cloning,
xii. Procedure for plating cells into soft agar medium (for soft agar method) or into 96 well plate (for microwell method),
    - For soft agar method, approximately 200 cells per plate in soft agar medium should be seeded in petri plates (designated as viable cell (VC) plates) and $1x10^6$ cells per plate in TFT-containing soft agar medium should be seeded in petri plates (designated as TFT plates) for mutant selection[237]. At a minimum, three plates per replicate sample (test and control) should be used for colony count for both VC plates and TFT plates.
    - For microwell method, approximately 1.6 cells per well in cloning medium should be seeded in 96 well plates (designated as viable cell (VC) plates) and approximately 2000 cells per well in TFT-containing cloning medium should be seeded in 96 well plates (designated as TFT plates) for mutant selection[238]. At a minimum, two 96-well plates per replicate sample (test and control) should be used for VC plates colony count and four 96-well plates per replicate sample (test and control articles) should be used for TFT plates colony count.
xiii. Procedure to determine optimal dose for testing (i.e., dose dependent study), if cytotoxicity is observed in any of the extracts,
xiv. Procedures for colony counting, colony sizing, and discrimination of small colonies vs. large colonies,
xv. Procedure for generation of historical negative (i.e., solvent/vehicle control) and positive control data (e.g., ranges, means and standard deviations), criteria for inclusion and exclusion of historical studies in the historical data cohorts, and how the expected range of mutant frequencies is established based on historical negative and positive control data[239]. The historical control data set should initially be built with at least 20 experiments conducted under comparable testing conditions, as used for medical device testing. The distribution of the data, together with appropriate descriptive statistics should be provided (e.g., confidence intervals, 95-99% percentiles),
xvi. Criteria for repeat testing,

[237] 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
[238] *Ibid*
[239] 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.

ISO/IEC 17025 Subclause 7.7(a)

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

i. The negative control meets the following criteria:

Parameter	Soft Agar Method	Microwell Method
Mutant Frequency (MF)	35 – 140 X 10^{-6}	50 – 170 X 10^{-6}
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)

ii. The positive controls meet at least one of the following two acceptance criteria for both 4-hr (with and without S9) and 24-hr (without S9) assays:
- The positive control demonstrates an absolute increase in total MF, that is, an increase above the spontaneous background MF. The increase in MF [i.e., the induced MF (IMF)] should be at least 300 X 10^{-6}. The small colony IMF should be compared to the total IMF, and should be at least 40% of the total IMF.
- The positive control has an increase in the small colony MF of at least 150 X 10^{-6} above that seen in the concurrent negative control (i.e., the small colony IMF of 150 X 10^{-6}).

iii. The relative total growth (RTG) of cells treated with positive controls should be 10% or greater.

B. Example ASCA Summary Test Report: In Vitro Mouse Lymphoma Test (ISO 10993-3 and OECD 490) — Soft Agar Method

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*[240]

☐ 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[241] (e.g., filtering, extract manipulation, pH adjustment):

Description of deviations/amendments

Extraction Solvent:

☐ RPMI medium (without serum)

☐ RPMI medium (with serum) *[Specify serum concentration per SOP and provide justification (e.g., all other non-polar solvents are not compatible with test article for extraction)]*

☐ 0.9% Saline *[Provide justification for using 0.9% Saline instead of RPMI medium without serum]*

☐ DMSO

☐ PEG 400 *[Provide justification for using PEG instead of DMSO (e.g., documentation of device degradation in DMSO)]*

[240] 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*.

[241] 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.

☐ Ethanol *[Provide justification for using ethanol instead of DMSO (e.g., documentation of device degradation in DMSO)]*

☐ Other:[242] *[Describe]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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)

*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]*

☐ Other:[243] *[Describe]*

Extraction Conditions:

☐ 37°C, 72 h

☐ 50°C, 72 h

☐ 70°C, 24 h

☐ 121°C, 1 h

☐ Other:[244] *[Describe]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[245]: *[Describe and provide justification]*

Fluid Path Extractions:

☐ 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:[246]

☐ Complete fill with agitation

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

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

☐ Other: *[Summarize approach]*

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[242] 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.

[243] 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.

[244] *Ibid*

[245] *Ibid*

[246] 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.

3327
3328

Test Article and Extract Appearance:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[247]

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.

ASCA Test Method SOP #: *[######-ASCAMLAAgar(date/version)]*

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[248]

Description of deviations/amendments

[247] 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.

[248] 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.

Results:

Test Article Extract and Control:

- **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]

- **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]

- **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]

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]

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]

Cell Density:

Suspension Growth Time Point	Initial Cell Density/Adjusted Cell Density
Day 0 (initial treatment day)	(e.g., 3×10^5 cells/mL)
Day 1 (one day after treatment)	
Day 2 (two days after treatment)	
Day 3 (three days after treatment)	

3354 **Table 1: Suspension Growth Data**

Suspension Growth Data (4-hour treatment without S9)					
Treatment Group	**[o]Replicate**	**Cell Density (× 10^5 cells/mL)**		**Suspension Growth ([□]SG)**	**Relative Suspension Growth ([†]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	**[o]Replicate**	**Cell Density (× 10^5 cells/mL)**		**Suspension Growth ([□]SG)**	**Relative Suspension Growth ([†]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	**[o]Replicate**	**Cell Density (× 10^5 cells/mL)**			**Suspension Growth ([◊]SG)**	**Relative Suspension Growth ([†]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
3356

[*]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 test article and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.

[º]At least 2 replicates should be used for each test article and control.

[¤] $SG_{4\ hr\ treatment} = \frac{Day\ 1\ cell\ density}{Initial\ cell\ density} \times \frac{Day\ 2\ cell\ density}{Adjusted\ cell\ density}$

[◊] $SG_{24\ hr\ treatment} = \frac{Day\ 1\ cell\ density}{Initial\ cell\ density} \times \frac{Day\ 2\ cell\ density}{Adjusted\ cell\ density} \times \frac{Day\ 3\ cell\ density}{Adjusted\ cell\ density}$

[†] $RSG = \frac{SG_{test\ article}}{SG_{control\ article}} \times 100\%$

3369 **Table 2: Viable Cell Plate (i.e., VC Plate) Colony Count Data**

VC Plate Colony Count Data (4-hour treatment without S9)								
Treatment	**[o]Replicate**	**Colony Count**			**Total Colony Count**	**Cloning Efficiency [n]$CE_{VC\ plate}$ (%)**	**Relative Cloning Efficiency †RCE (%)**	**Relative Total Growth ◊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	**[o]Replicate**	**Colony Count**			**Total Colony Count**	**[n]$CE_{VC\ plate}$ (%)**	**†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							

3371

VC Plate Colony Count Data (24-hour treatment without S9)								
Treatment	**ºReplicate**	**Colony Count**			**Total Colony Count**	**¤$CE_{VC\ plate}$ (%)**	**†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							

*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 test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.
ºAt least 2 replicates should be used for each test article and control.

$$\text{◊}\ RTG = RSG \times RCE$$

$$\text{¤}CE_{VC\ plate} = \frac{Total\ Colony\ Count/3}{Initial\ number\ of\ cells\ seeded\ in\ VC\ plate} \times 100\%$$

$$\text{†}\ RCE = \frac{CE_{test\ article}}{CE_{control\ article}} \times 100\%$$

Table 3: Selective Plate (i.e., TFT Plate) Colony Count Data

TFT Plate Colony Count Data (4-hour treatment without S9)														
Treatment	**Replicate**	**Colony Counts**						**$^{\times}N_{small}$**	**$^{\times}N_{total}$**	**◊CE_{TFT}**	**†MF**	**¤scMF**	**˙IMF**	**‡scIMF**
		Plate 1		**Plate 2**		**Plate 3**								
		$^{\times}$S	**$^{\times}$L**	**$^{\times}$S**	**$^{\times}$L**	**$^{\times}$S**	**$^{\times}$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													

3388

TFT Plate Colony Count Data (4-hour treatment with S9)														
Treatment	**Replicate**	**Colony Counts**						**×N_{small}**	**×N_{total}**	**◊CE_{TFT}**	**†MF**	**¤scMF**	**·IMF**	**‡scIMF**
		Plate 1		**Plate 2**		**Plate 3**								
		×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													

3389
3390

TFT Plate Colony Count Data (24-hour treatment without S9)														
Treatment	**Replicate**	**Colony Counts**						**$^{\times}N_{small}$**	**$^{\times}N_{total}$**	**◊CE_{TFT}**	**†MF**	**▫scMF**	**·IMF**	**‡scIMF**
		Plate 1		**Plate 2**		**Plate 3**								
		$^{\times}$S	**$^{\times}$L**	**$^{\times}$S**	**$^{\times}$L**	**$^{\times}$S**	**$^{\times}$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													

*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 (e.g., MMS) in the result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.

ºAt least 2 replicates should be used for each test article and control.

×S: small colony; L: large colony; N_{small}: total small colony count; N_{total}: total colony count.

◊ $CE_{TFT\ plate} = Cloning\ Efficiency\ of\ TFT\ plate = \frac{Total\ Colony\ Count/3}{Initial\ Number\ of\ Cells\ Seeded\ in\ TFT\ Plate}$

† $MF = Mutant\ Frequency = \frac{CE_{TFT\ plate}}{CE_{VC\ plate}} \times 10^{-6}$

▫ $scMF = Small\ Colony\ Mutanty\ Frequency = MF \times \frac{Total\ Small\ Colony\ Count}{Total\ Colony\ Count} \times 100\%$

· $IMF = Induced\ Mutant\ Frequency = MF_{test\ article\ or\ positive\ control} - Mean\ MF_{negative\ control}$

‡ $scIMF = Small\ Colony\ Induced\ Mutant\ Frequency = scMF_{test\ article\ or\ positive\ control} - Mean\ scMF_{negative\ control}$

‡scIMF data are needed for positive controls and test articles if test articles demonstrate a positive response.

I confirm that the following test validity criteria are met[249]:

☐ The negative control meets all following criteria:

- Mutant Frequency (MF): 35 – 140 per 10^6 cells
- Cloning Efficiency (CE): 65 – 120%
- Suspension Growth (SG): 8-32 fold (3-4 hour treatment), 32-180 fold (24-hour treatment)

☐ 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:

- 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^{-6}. In addition, the small colony IMF is at least 40% of the total IMF.
- 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^{-6}.

☐ The relative total growth (RTG) of cells treated with the positive controls and test article extracts are 10% or greater (i.e., RTG ≥ 10%)

Overall Results:

☐ 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) [250] of 90 x 10^{-6}.

☐ The test article demonstrated a positive response[251]. 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^{-6} AND the RTG of cells treated with the test article extracts is 10% or greater.

☐ 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)[252].

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.

[249] 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.

[250] 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.

[251] 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.

[252] *Ibid*

3435

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

C. Example ASCA Summary Test Report: In Vitro Mouse Lymphoma Test (ISO 10993-3 and OECD 490) —Microwell Method

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*[253]

☐ 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[254] (e.g., filtering, extract manipulation, pH adjustment):

Description of deviations/amendments

Extraction Solvent:

☐ RPMI medium (without serum)

☐ RPMI medium (with serum) *[Specify serum concentration per SOP and provide justification (e.g., all other non-polar solvents are not compatible with test article for extraction)]*

☐ 0.9% Saline *[Provide justification for using 0.9% Saline instead of RPMI medium without serum]*

☐ DMSO

☐ PEG 400 *[Provide justification for using PEG instead of DMSO (e.g., documentation of device degradation in DMSO)]*

[253] 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*.

[254] 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.

☐ Ethanol *[Provide justification for using ethanol instead of DMSO (e.g., documentation of device degradation in DMSO)]*

☐ Other:[255] *[Describe]*

Extraction Ratio:

☐ 6 cm^2/ml (<0.5 mm thick)*

☐ 3 cm^2/ml (0.5-1.0 mm thick or molded items > 1.0 mm thick)*

☐ 1.25 cm^2/ml (elastomers > 1.0 mm thick)*

☐ 0.2 g/ml (for powder devices)

☐ 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)

*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]*

☐ Other:[256] *[Describe]*

Extraction Conditions:

☐ 37°C, 72 h

☐ 50°C, 72 h

☐ 70°C, 24 h

☐ 121°C, 1 h

☐ Other:[257] *[Describe]*

Agitation During Extraction:

☐ Extraction with continuous agitation or circulation

☐ Extraction under static conditions or intermittent agitation[258]: *[Describe and provide justification]*

Fluid Path Extractions:

☐ 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:[259]

☐ Complete fill with agitation

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

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

☐ Other: *[Summarize approach]*

[255] 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.

[256] *Ibid*

[257] *Ibid*

[258] *Ibid*

[259] 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.

Test Article and Extract Appearance:

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

☐ There were changes in color/turbidity or particles in the test article and/or extract OR there was swelling/degradation of the test article.[260]

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.

ASCA Test Method SOP #: *[######-ASCAMLAMicrowell(date/version)]*

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

☐ Test was conducted per the above protocol and 21 CFR 58, with the following deviations/amendments:[261]

Description of deviations/amendments

[260] 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.

[261] 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.

Results:

Test Article Extract and Control Dose:

a. **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]

b. **Test Article Extract:**

Test Article Extract	Treatment Dose
[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]

c. **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]

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]

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]

Cell Density:

Suspension Growth Time Point	Cell Density/Adjusted Cell Density
Day 0 (initial treatment day)	(e.g., 3×10^5 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)	

Table 1: Suspension Growth Data

Suspension Growth Data (4-hour treatment without S9)					
Treatment Group	**[o]Replicate**	**Cell Density (× 10^5 cells/mL)**		**Suspension Growth ([□]SG)**	**Relative Suspension Growth ([†]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	**[o]Replicate**	**Cell Density (× 10^5 cells/mL)**		**Suspension Growth ([□]SG)**	**Relative Suspension Growth ([†]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	**[o]Replicate**	**Cell Density (× 10^5 cells/mL)**			**Suspension Growth ([◊]SG)**	**Relative Suspension Growth ([†]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					

*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 (e.g., MMS) in the result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.

ºAt least 2 replicates should be used for each test article and control.

¤ $SG_{4\ hr\ treatment}=\frac{Day\ 1\ cell\ density}{Initial\ cell\ density}\times\frac{Day\ 2\ cell\ density}{Adjusted\ cell\ density}$

◊ $SG_{24\ hr\ treatment}=\frac{Day\ 1\ cell\ density}{Initial\ cell\ density}\times\frac{Day\ 2\ cell\ density}{Adjusted\ cell\ density}\times\frac{Day\ 3\ cell\ density}{Adjusted\ cell\ density}$

† $RSG=\frac{SG_{test\ article}}{SG_{control\ article}}\times 100\%$

3546 **Table 2: Viable Cell Plate (i.e., VC Plate) Colony Count Data**
3547

VC Plate Colony Count Data (4-hour treatment without S9)								
Treatment	**ºReplicate**	**Colony Counts**		**×N_{TW}**	**×N_{EW}**	**Cloning Efficiency ¤$CE_{VC\ plate}$ (%)**	**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							

3548

VC Plate Colony Count Data (4-hour treatment with S9)								
Treatment	**ºReplicate**	**Colony Counts**		**×N_{TW}**	**×N_{EW}**	**¤$CE_{VC\ plate}$ (%)**	**†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							

VC Plate Colony Count Data (24-hour treatment without S9)								
Treatment	**ºRep.**	**Colony Counts**		**×N_{TW}**	**×N_{EW}**	**▫$CE_{VC\ plate}$ (%)**	**†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							

*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 (e.g., MMS) in the result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.

ºRep.=replicate. At least 2 replicates should be used for each test article and control.

× N_{TW}=total number of wells with colonies, N_{EW}= total number of empty wells.

$$▫CE_{VC\ plate} = \frac{-Ln(\frac{N_{EW}}{2 \times 96})}{Initial\ number\ of\ cells\ seeded\ in\ VC\ plate}$$

$$† RCE = Releative\ Coloning\ Efficiency = \frac{CE_{test\ article}}{CE_{control\ article}} \times 100\%$$

$$◊ RTG = RSG \times RCE$$

Table 3: Selective Plate (i.e., TFT Plate) Colony Count Data

TFT Plate Colony Count Data (4-hour treatment without S9)																	
Treatment	**[o]Rep.**	**Colony Counts**								**[×]N_{TW}**	**[×]N_S**	**[×]N_{EW}**	**[◊]CE_{TFT}**	**†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																

3565

TFT Plate Colony Count Data (4-hour treatment with S9)																	
Treatment	**[o]Rep.**	**Colony Counts**								**[×]N_{TW}**	**[×]N_S**	**[×]N_{EW}**	**◊CE_{TFT}**	**†MF**	**[n]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																

3566
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TFT Plate Colony Count Data (24-hour treatment without S9)																	
Treatment	**◦Rep.**	**Colony Counts**								**×N_{TW}**	**×N_S**	**×N_{EW}**	**◊CE_{TFT}**	**†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																

3569
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[×]S=small, L=Large, N_{TW}=total number of wells with colonies, N_{EW}= total number of empty wells, Ns=total number of wells with small colonies.

[º]Rep.=replicate. At least 2 replicates should be used for each test article and control.

*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 (e.g., MMS) in the result table. For test articles and positive controls, two or more concentrations may be used. If so, include a separate row for each concentration.

◊ $\mathrm{CE_{TFT\ plate}} = \dfrac{-\ln\dfrac{\mathrm{N_{EW}}}{4\times 96}}{\text{Initial number of cells seeded per well in TFT plate}}$

† $\mathrm{MF} = \text{Mutant Frequency} = \mathrm{MF} = \dfrac{\mathrm{CE_{TFT\ plate}}}{\mathrm{CE_{VC\ plate}}} \times 10^{-6}$

[¤]$\mathrm{scMF} = \text{Small Colony Mutant Frequency} = \mathrm{MF} \times \dfrac{\mathrm{N_S}}{\mathrm{N_{TW}}} \times 100\%$

·$\mathrm{IMF} = \text{Indusced Mutant Frequency} = \mathrm{MF_{test\ article\ or\ positive\ control}} - \text{Mean}\ \mathrm{MF_{negative\ control}}$

‡$\mathrm{scIMF} = \text{Small Colony Mutant Frequency} = \mathrm{scMF_{test\ article\ or\ positive\ control}} - \text{Mean}\ \mathrm{scMF_{negative\ control}}$

‡scIMF data are needed for positive controls and test articles if test articles demonstrate a positive response.

I confirm that the following test validity criteria are met[262]:

☐ The negative control meets the following criteria:

- Mutant Frequency (MF): 50 – 170 per 10^6 cells
- Cloning Efficiency (CE): 65 – 120%
- Suspension Growth (SG): 8-32 fold (3-4 hour treatment), 32-180 fold (24-hour treatment)

☐ For both 4-hr (with and without S9) and 24-hr (without S9) assays, the positive control(s) meet at least one of the following criteria:

- ☐ The positive control demonstrated an absolute increase in total MF, that is, an increase above the spontaneous background MF, and this increase in MF [i.e., the induced MF (IMF)] is at least 300 X 10^{-6}. In addition, the small colony IMF is at least 40% of the total IMF.
- ☐ The positive control demonstrated an increase in the small colony MF above the concurrent negative control and the increase in the small colony MF (i.e., the small colony IMF) is at least 150 X10^{-6}

☐ The relative total growth (RTG) of cells treated with the positive controls is 10% or greater (i.e., RTG ≥ 10%)

Overall Results:

☐ 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) [263] of 126 x 10^{-6}.

☐ The test article demonstrated a positive response[264]. The results are positive if under any experimental conditions (i.e., 4-hr treatment with and without S9 fraction, 24-hr treatment without S9 fraction) examined, the IMF in any test article extract exceeds the Global Evaluation Factor (GEF) of 126 x 10^{-6} AND the RTG of cells treated with the test article extracts is 10% or greater.

☐ 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)[265].

[262] 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.

[263] 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.

[264] 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.

[265] *Ibid*

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.

3616

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