



DEPARTMENT OF HEALTH & HUMAN SERVICES

U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Compliance and Biologics Quality
Division of Manufacturing and Product Quality

To: Administrative Files: STN 125348/0 for Isolagen Therapy™

From: Randa Melhem, Ph.D., OCBQ, DMPQ, MRBII, HFM-676

cc: Gang Wang, Ph.D., OCBQ, DMPQ, MRBII, HFM-676

Through: David Doleski, Acting Branch Chief, OCBQ, DMPQ, MRB II, HFM-676

Subject: **Review Memo (BLA):** [previously Isolagen Technologies, Inc., currently Fibrocell Science Inc. – US License 1818]: Review of original Biologics License Application (BLA) for the autologous human fibroblast cell therapy (previously Isolagen Therapy™ (IT), currently azfibrocel-T), for the treatment of moderate to severe nasolabial fold wrinkles, manufactured at their Exton, PA facility.

Action Due: January 4, 2010

Action Recommended

There are deficiencies in CMC section of the submission. The proposed letter-ready comments for the CR letter are as follows:

Inspectional Issues

1. Outstanding inspectional issues from the pre-license inspection conducted on August 31 to September 4, 2009, have yet to be resolved.

Container Closure

2. Regarding the Container Closure Integrity Testing (CCIT) method:
 - a. The sensitivity of the method has not been validated. Please provide such data.
 - b. Please submit CCIT data generated after freezing and thawing of the container closure to simulate freezing of the Drug Substance-Cryovial.
3. The container closure -----(b)(4)----- in the initial and confirmatory testing. The cryovials tested -----(b)(4)----- than the ----(b)(4)---- specified limit of -----(b)(4)----- . Please explain

the corrective actions that have been implemented to address this issue along with data for the final container that is within the limits described by the applicable (b)(4) test methods.

Shipping Validation Studies

4. Shipping validation studies performed under protocol EX-PRT-116 and EX-PRT-121 have failed to demonstrate that the shippers can maintain the specified range of shipping temperatures. In addition, the studies have failed to demonstrate that the shipping process is able to maintain the stability of Drug Product Injection for 48 hours. Please describe your actions to address this concern, along with validation data to demonstrate that temperatures can stay within specified ranges and that the product will remain stable for 48 hours.

SUMMARY / HISTORY

Isolagen Technologies, Inc. (Isolagen) submitted this original BLA on March 6, 2009. This application is a paper submission in CTD format and consists of five (5) modules. In addition, electronic SAS data files and programs are being submitted on a single CD-ROM in Module 1. In addition the Package Insert is provided as a Word document (.doc) and in SPL format on a single CD-ROM. The eCRFs with appropriate bookmarks are provided on two CD-ROMs.

Isolagen proposes in this BLA for the use of autologous human fibroblasts (Isolagen Therapy™) for the treatment of moderate to severe nasolabial fold wrinkles. Isolagen has conducted 7 clinical trials to support this clinical indication under BB-IND -(b)(4)-.

Isolagen Therapy™ (IT) cell therapy product is composed of a suspension of autologous fibroblasts, grown from a biopsy of each individual's own skin (dermis and epidermis) and expanded to a quantity sufficient for injection into the patient's target treatment area.

Isolagen Inc. has changed its name to Fibrocell Science Inc. and changed the name of its main product Isolagen Therapy™ to azfibrocel-T.

SUBMISSION CONTENT

This paper submission is comprised of 5 modules:

Module 1: Administrative information and Prescribing Information

Module 2: Common Technical Document Summaries (Executive Summary)

Module 3: Quality

3.2.S Drug Substance

3.2.P Drug Product

3.2.A Appendices

Module 4: Nonclinical

Module 5: Clinical Study Reports

SUBMISSION REVIEW

In this review, I will cover the manufacturing process, and process validation, the container closure system, and facilities and equipment.

Introduction

Isolagen Therapy™ (IT) consists of autologous human fibroblasts used for the treatment of moderate to severe nasolabial fold wrinkles. It consists of three injection treatments. Drug Product material prepared for each injection treatment is defined as a batch. For the treatment of nasolabial fold wrinkles, a single injection treatment (batch) requires two 2 mL vials containing 1.2 mL/vial of IT Drug Product-Injection.

Isolagen Therapy™ (IT) Drug Substance consists of a suspension of fibroblasts in cryopreservation medium containing (b)(4) dimethyl sulfoxide (DMSO). The Drug Substance is stored in aliquots in cryovials in the vapor phase of a liquid nitrogen freezer until preparation of the IT Drug Product.

The Isolagen Therapy™ (IT) Drug Product - Injection consists of a suspension of living autologous fibroblasts formulated to a concentration of $1.0-2.0 \times 10^7$ cells/mL in Dulbecco's Modified Eagle's Medium (DMEM) without phenol red. The formulated Drug Product is filled at a volume of 1.2 mL per container immediately prior to shipment to the clinical site of patient administration. Two Drug Product - Injection vials are prepared and shipped per treatment administration.

All commercial in-process and release testing of the Bulk Harvest and Drug Substance Cryovial is performed by Isolagen's Exton facility, with the exception of mycoplasma testing which will be outsourced to -----(b)(4)-----

Manufacture of Isolagen Therapy™ for Phase 3 clinical use has been conducted at Isolagen's Exton, PA facility (FDA Registration Number 3005836954) since October 2006. Over (b)(4) clinical lots of IT have been manufactured in the Exton facility for all active INDs (INDs -----(b)(4)-----). Commercial manufacture of IT will be conducted in the same facility using the same process, segregation and changeover procedures, and analytical methods developed for manufacture of clinical lots.

3.2.S.1 - Drug Substance

Isolagen Therapy™ Drug Substance consists of a suspension of fibroblasts in cryopreservation medium containing (b)(4) Dimethyl sulfoxide (DMSO). The Drug Substance is stored in aliquots in cryovials in the vapor phase of a liquid nitrogen freezer until preparation of the IT Drug Product.

The autologous fibroblasts in the (IT) Drug Substance are derived by outgrowth from a biopsy of the recipient's own skin followed by expansion in culture using standard cell culture techniques. At the completion of culture expansion, the cells are harvested and washed, then formulated to contain ----(b)(4)----- cryopreserved in cryovials. The Drug Substance is tested for purity and confirmed to contain $\geq 98\%$ fibroblasts. Viability of the cells must be --(b)(4)- for product release. Sterility and endotoxin testing are also conducted during release testing of Drug Substance - Cryovial using the procedure described in --(b)(4)---- and --(b)(4)---- .

The cells in the Drug Substance formulation display typical fibroblast morphologies when growing in cultured monolayers. The cells express proteins characteristic of normal

fibroblasts including the fibroblast -----(b)(4)-----
-----, and the extracellular matrix protein, collagen.

3.2.P.1 – Drug Product

The Isolagen Therapy™ (IT) Drug Product - Injection consists of a suspension of living autologous fibroblasts formulated to a concentration of 1.0-2.0 x 10⁷ cells/mL in Dulbecco's Modified Eagle's Medium (DMEM) without phenol red.

The primary packaging component for Drug Product- Injection is 2mL self-standing, round-bottom vial with an externally threaded screw cap closure fitted with a -(b)(4)--- washer -----(b)(4)-----.

3.2.A.1 FACILITIES AND EQUIPMENT

3.2.A.1.1 Facility Overview

Manufacture of the Drug Substance-Cryovial for commercial product will be performed at Isolagen's Exton, PA facility. The Exton facility is a -----(b)(4)----- square foot, aseptic autologous cellular therapy manufacturing building, located at 405 Eagleview Blvd. in Exton, PA. The process area is comprised of Shipping and Receiving, Warehousing, the Process Suite, and the Quality Control Laboratories. All cellular processing is done inside -----(b)(4)-----, which are located inside a -----(b)(4)----- Cleanrooms.

The Exton facility has multiple entrances and a service dock, which facilitates the control of incoming and outgoing material flow and personnel traffic in the office, laboratory, and manufacturing areas to support CGMP operations as well as office and laboratory activities. Part of the building has been renovated as the -----(b)(4)-----.

However, the existing boilers, chillers, water supply, sanitary and power capacity support these functions without substantial alteration. The existing emergency generators are capable of providing the required emergency power to the production operations.

Isolagen describes the different areas of the facility and provides a list of the manufacturing and support areas as shown below:

[(b)(4)]

[(b)(4)]

[(b)(4)]

3.2.A.1.4 Facility Support System

Water Systems

Isolagen states that purified water or Water for Injection (WFI) systems are not used in the manufacture of Isolagen Therapy™. All water used in the manufacture of (IT) is purchased. WFI, which complies with the (b)(4) monograph for WFI, is used for --- (b)(4) -- cleaning, and distilled water is used for ----- (b)(4) ----- . The deionized (DI) water system at Isolagen is a ----- (b)(4) ----- .

Carbon Dioxide (CO₂)

Carbon dioxide in compliance with the (b)(4) monograph is delivered in charging containers by qualified vendors for supply use to -- (b)(4) -- . The charging containers are connected to --- (b)(4) -- located in the CGMP utility room for controlled distribution within the facility. The distribution piping connects to ----- (b)(4) ----- , as

Facility Monitoring

The -----(b)(4)----- monitors the facility and equipment deemed critical to the manufacturing process. These parameters include room temperature, humidity, and differential pressure and equipment temperatures, CO₂ levels, and door alarms.

3.2.A.1.5 Critical Equipment

Isolagen provides a summary of the manufacturing equipment and status of equipment qualification as shown in the following table

[(b)(4)]

3.2.A.1.6 Prevention of Cross contamination

Isolagen states that they put in place procedures and controls to prevent cross-contamination; these include training of personnel in aseptic practices, cleaning and sanitization of equipment and use of disposable -----(b)(4)----- . Quality control practices include visual observations by manufacturing staff, and QC tests for sterility, mycoplasma, endotoxin and Gram stain.

They explain that Biopsies are processed under aseptic conditions and controlled utilizing a unique Part and Lot number for each biopsy to prevent cross contamination. All -----

The cap-inserts fit into the exterior well on the top of the vial cap and have no contact with the contents of the vial.

The formulated Drug Product is filled at a volume of 1.2 mL per container immediately prior to shipment to the clinical site of patient administration. Two Drug Product - Injection vials are prepared and shipped per treatment administration.

The dimensions of the container closure are as follows:

Parameter	Dimension
Vial outer diameter	12.41 mm
Vial Inner diameter	10.01 mm
Length of vial with cap	48.54mm
Length of vial without cap	41.89 mm

The vial, cap and washer all meet the -----(b)(4)----- requirements for plastic containers and closures.

Isolagen states that the container closure system conforms to the physical standards for plastic materials and components used to package medical articles, as specified in – (b)(4)---. The identity and characterization of the polypropylene composing the container closure was established by -----(b)(4)-----, conducted per Isolagen protocol EX-PRT-117, *Final Container Closure Testing*. The tests were performed by -----(b)(4)----- . Isolagen provides the test results in EX-GTR-117, *Final Container Closure Testing*, as well as -----(b)(4)----- Certificate of Analysis.

They add that all tests met the defined acceptance criteria with the exception of the test for -----(b)(4)----- . The out of specification results of the original -----(b)(4)----- and the confirmatory tests using freshly prepared -----(b)(4)----- exceeded the -----(b)(4)----- . Isolagen justified the result as being due to the exterior surface of the vial which is printed with volume gradations in black ink and a large white frosted area for labeling. Isolagen adds that the Drug Product - Injection formulation does not include any organic solvents, and thus they considered the results acceptable.

Reviewer’s comments: Additional information was requested to address the high hexane results, and the firm stated that additional testing will be done as described in response to **Q9** reviewed in the Information Request section below.

The caps and vials are –(b)(4)--- sterilized and are received sterile from the manufacturer. The specifications for the container closure system are described in document RMS-5038. The cryovials are released based on the manufacturer's Certificate of Compliance which lists the acceptance criteria and tests performed.

[(b)(4)]

will be performed to address the container closure integrity testing. The information is reviewed in Q10 of the Information Request section below.

Biological Reactivity

Isolagen states that the biological reactivity of container closure extracts was assessed according to the method described in ---(b)(4)--, and was performed by -----
----- (b)(4)-----

----- (b)(4)-----

Isolagen concludes that the container closure is suitable for Drug Product-Injection under the conditions used for shipment and storage of the product at the clinical site.

Short-term storage – Stability of Drug Product-Injection

Isolagen states that in three separate studies (protocols EX-PRT-013 v01, EX-PRT-013 v02 and EX-PRT-112 v00), Drug Product - Injection vials were found to remain within specification for all release criteria for at least 48 hours of storage at 2-8°C. In a fourth study (EX-PRT-086), where the stability of the Drug Product was tested after 24 hours of storage at 2-8°C followed by ----(b)(4)----- of storage at room temperature to simulate potential conditions of storage in the clinic, the Drug Product was found to remain within specification for cell count and viability for up to --(b)(4)--- of storage at room temperature.

Isolagen concludes that the container closure is suitable for maintaining sterility, viability and other quality attributes of the Drug Product during storage.

Suitability of Secondary packaging/Shipping

Two Drug Product-Injection vials are placed in an upright position into an inner biohazard leak-proof poly bag containing a 50 mL absorbent strip. The biohazard bag is then placed into a Dupont Tyvek® envelope. This tamper-evident secondary packaging is used to contain any possible spillage occurring during transit. The Dupont Tyvek® envelope containing the inner biohazard leak-proof poly bag will be placed in the shipping container immediately prior to shipment.

The shipping container consists of an outer box enclosing a polystyrene cooler with lid. Within the polystyrene cooler, an inner box is positioned between two previously-frozen freezer blocks composed of clear hard plastic, one on each side. Phase change material -(b)(4)- soft gel packs will be placed in front of and behind the inner box, two on each side The -(b)(4)- gel packs ----- (b)(4)----- . A

minimum of two 12" x 12" sheets of bubble wrap are folded into quarters and placed in the inner box. The assembled shipping container with the polystyrene lid in place is preconditioned to 2-8°C for at ---(b)(4)-----.

Isolagen performed shipping validation studies to evaluate the suitability of the secondary packaging system to maintain the quality attributes of Drug Product - Injection during shipment to the clinical site (3.2.P.3.5 App 10, 11 14 and 15).

In EX-GTR-116 report, Isolagen states that some of shipping containers did not maintain the specified temperature of 2-8°C, particularly during the first 24 hours of data collection during which temperatures moved slightly above or below the specified window. In addition, not all of the Drug Product samples remained within specification for cell count and viability at the end of the 48 period.

Additional validation studies were performed to include a specified period of --(b)(4)-----(b)(4)- during which the lid of the shipper was removed. In addition, this study limited the time period during which the Drug Product remained in transit to 24 hours which more accurately reflects the shipment of IT during commercial production. In EX-GTR-121, Isolagen states that the studies verified the suitability of this shipper and the selected mode of transport for shipping of Drug Product – Injection material to clinical sites.

Reviewer’s comments: The data provided in EX-GTR-121 show excursions outside the acceptance criteria. In addition one of the three lots failed to meet specifications for cell count after 48 hrs. Additional information was requested. The information was provided in amendment 125348/ 0.20 and reviewed in the Information request section **Q11**. The Secondary Packaging/Shipping is also described in more detail in the Product Review Memo.

3.2.P.2.5 Microbiological Attributes

Isolagen states that the Isolagen Therapy™ Drug Product-Injection is manufactured aseptically using CGMPs and tested for microbiological purity at -----(b)(4)---- in the manufacturing process. The Drug Product-Injection is shipped overnight to the clinical site, to be injected within 48 hours of preparation.

Stability testing of Drug Product-Injection has demonstrated loss of viability beyond 48 hours (EX-PRT-013, *Stability Study for Drug Product-Injection*), leading to the expiration dating of product 48 hours beyond injection preparation. All testing met the same specifications used for product release.

Because of this short shelf-life, results of sterility testing done on Drug Product-Injection are received after the injection occurs. Isolagen states that sterility testing is performed on the Drug Substance-Cryovial, and a Gram stain assay (ATM-001) is used for provisional sterility screening of Drug Product-Injection before release. The firm confirms that no product in the pivotal Studies IT-R-005 or IT-R-006 showed positive results on a Gram stain or sterility test conducted on injection material.

The microbiological testing on the Drug Product-Injection is described below. This includes in-process testing of Bulk Harvest, drug substance release testing on Drug Substance-Cryovial and drug product release testing on Drug Product-Injection.

6 pages redacted due to (b)(4)

2. -----

-(b)(4)-

-(b)(4)-

3.2.P.3.5 (App. 8 and 9) Isolagen Standardized Manufacturing Process Validation Protocol (EX-PRT-110, EX-GTR-110)

Isolagen states that this validation protocol outlines the approach, experiments and data analysis of Isolagen manufacturing process for consistently producing Bulk Drug Substance - Cryovial and Drug Product - Injection lots that meets the acceptance criteria during multiple manufacturing runs. The validation protocol is executed to:

- Demonstrate that the manufacturing process will reliably and reproducibly produce Drug Substance and Drug Product that meets the defined in-process and final control and release specifications.
- Demonstrate the consistency of manufacture produced by the commercial process at the current and planned launch commercial maximum capacity (biopsies/day) for the Exton facility.

-(b)(4)-

-(b)(4)-

- ------(b)(4)-----:

-(b)(4)-

5 pages redacted due to (b)(4)

Stability Studies

Isolagen states that both the Bulk Drug Substance - Cryovial and Drug Product - Injection stages of lots prepared for EX-PRT-110 were placed on stability studies. Stability data is reviewed in the Product Review memo.

Additional Processing (Step 7a)

Isolagen states that protocol EX-PRT-110 permitted the use of Step 7a in the event full yield could not be achieved by the end of the primary culture process (end of P3). However, additional processing was not required during the execution of the study since all validation lots achieved yield at the end of primary culture. However, an additional processing run was performed on one lot from the study (DR01 200811463, assigned an "a" for the first additional processing replicate) to support the use of Step 7a in the process to produce additional cells. Isolagen presents the summary data for the growth specifics testing results of the Bulk Drug Substance – Cryovial. All results were within specifications.

Isolagen reports that the additional processing culture generated sufficient Bulk Drug Substance vials to provide an additional nasolabial fold treatment (six cryovials containing 1.2 mL Drug Product - Injection), with additional material left in storage. The culture met all process parameters, including confluence and days in culture. These results suggest that even when an additional passage is employed to generate additional cells, an acceptable yield can be obtained with high viability.

Deviations

Isolagen reports two deviations that occurred during the execution of the protocol. Both deviations were attributed to clerical errors and do not impact the data generated during the execution of EX-PRT-110.

Conclusion

Isolagen concludes that all acceptance criteria for critical control parameters, in-process testing and all completed release and stability testing were met for EX-PRT-110. A series of (b)(4) production lots were simultaneously initiated and processed, and have successfully completed production and revealed adequate results to meet the requirements for product release. They note that there was variability between lots for some process parameters, but this is expected since it is an autologous product consisting of primary culture from tissues of different sources.

Reviewer’s comments: During the PLI, Isolagen committed to manufacture ---(b)(4)----
----- and not more than -----(b)(4)----- for their soft launch of the product.

2 pages redacted due to (b)(4)

[(b)(4)]

-----(b)(4)-----

Reviewer's comments: Response is acceptable

2. The media fills conducted under EX-PRT-120 and EX-GTR-120 only covered the fill of the Bulk Drug Substance-Cryovial and the Drug Product-Injection stages of the Isolagen Process. Media fills should also cover other aseptic processes and simulate worst case scenarios. Please provide the SOPs for media fills, the conditions for worst case scenarios, and data to support the state of control in worst case.

Isolagen reiterates their plan to perform full process simulation media fills in December 2009, including worst-case scenarios in number of vials filled according to SOP-MA-021, *Media Fill Qualification (Draft)*.

They state that all process parameters with time ranges are simulated at their maximum end of ranges.

[(b)(4)]

Isolagen states that they will perform interventions (dictated by QC) to simulate unanticipated occurrences during processing of Isolagen Therapy™ that can stress the aseptic nature of the process. Environmental monitoring is also performed during the media fill simulations. QC is responsible for collecting observations, ensuring the completion of all interventions, and completing environmental monitoring.

Isolagen provides the acceptance criteria for the media simulation:

- -----(b)(4)-----

- -----(b)(4)-----
- -----(b)(4)-----

- -----

----- (b)(4) -----

Reviewer's comments: During the PLI, the firm was cited (483 observation #2) for the inadequacy of the media-fill studies. The proposed study will be reviewed in the 483 response memo.

Note: This issue is closed from a review standpoint.

3. You state that Water-for-Injection (WFI), distilled water, carbon dioxide and liquid nitrogen are purchased. Please list the qualified vendors, and describe the tests (and frequency of testing) used to assure the quality of the purchased products.

Isolagen provides the vendor and release criteria for WFI, distilled water, carbon dioxide and liquid nitrogen as summarized in the following Table

[(b) (4)]

----- (b)(4) -----

----- (b)(4) -----

Reviewer's comments: During the PLI, the firm stated that they do not perform any testing for bioburden of the ----- (b)(4) ----- . They also stated that they --(b)(4)- ----- when needed and ----- (b)(4) ----- . This was a discussion item which is described in the EIR. *Note: This issue is closed from a review standpoint.*

-4. Please provide the protocol and discuss the results for testing ---(b)(4)----- and differential pressure at the Isolagen facility.

----(b)(4)---:

----- (b)(4) -----

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----- (b)(4) -----

- -----
----- (b)(4) -----

- -----
----- (b)(4) -----

- -----
----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

Differential Pressure

Isolagen states that differential pressure for classified and surrounding manufacturing and testing areas at the Exton facility is measured using calibrated pressure sensors that report results to the ----(b)(4)----- The ---(b)(4)--- System is a computerized system that collects, reports and archives real-time environmental conditions. --(b)(4)--- is automated and runs continuously without the need for manual manipulation or readings by Isolagen personnel. The system

2 pages redacted due to (b)(4)

Alert and action levels are re-evaluated regularly by reviewing the trend reports against current industry trends and standards according to SOP-EM-002, *Establishing Viable Environmental Monitoring Alert and Action Levels*.

Reviewer's comments: The EM- SOPs provide the frequency of monitoring for both viable and non viable particles, and the methods used for monitoring. The SOPs do not include validation studies to determine worst case locations and number of samples needed per area. During the PLI, the firm was cited (483 observation #6) for the inadequacy of the EM performance qualifications. The EM validation will be reviewed in the 483 response memo.

Note: This issue is closed from a review standpoint.

6. Please provide cleaning and EM validation studies performed during a facility shutdown and the process by which the facility is brought back up following a shutdown.

Isolagen states that they performed study EX-GTR-085, '*Disruption to Controlled Environments Study for -----(b)(4)----- areas*'. Based on the results of these studies, Isolagen drafted SOP-QA-017 '*Responding to Disruptions to Controlled Environments (Draft)*' describing the process they use at Exton facility to bring the facility back up following a shut down (Appendix 10).

In case of a disruption (or a shut down), the cleaning and disinfection performed follows the -(b)(4)- regimen, as described in SOP-MA-012, *Cleaning and Disinfection Program of Aseptic and Support Areas*. This cleaning and disinfection -----(b)(4)----- in all affected rooms or (b)(4). At a minimum, -(b)(4)-- of cleaning, disinfection and environmental monitoring is performed; however, -----(b)(4)----- may be required, as determined by the Head of Quality, based upon the severity and duration of the DCE.

Reviewer's comments: There are no validation studies of worst case scenario, so it is not clear if the --(b)(4)--- cleaning with ---(b)(4)--- would be enough. The SOP is not very specific with regards to cleaning, EM or additional testing following a shut down or disruption. During the PLI, the firm was cited (483 observations # 5 and #6) for the lack of cleaning validations and the inadequacy of the EM performance qualifications. The cleaning and EM validation studies will be reviewed in the 483 response memo.

Note: This issue is closed from a review standpoint.

7. In the BLA, you provide a brief description of the cleaning and disinfection of the facility and equipment. We require the following additional information:

a. The validation study for the cleaning and disinfection of the facility including selection of method of cleaning/disinfection, frequency and cleaning/disinfecting agent.

Isolagen states that they are planning to conduct disinfectant effectiveness studies on the disinfectants intended for use during commercial manufacturing operations. Each disinfectant and --(b)(4)- will be subjected to time-kill studies for microorganisms -----(b)(4)----- representing surfaces found in the Isolagen Cleanroom environment, in accordance with ----(b)(4)---- and FDA Guidance for Industry: *Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice*

(b)(4)

b. The routine cleaning/disinfection protocol for the facilities.

Isolagen included in Appendix 9 SOP-MA-012, *Cleaning and Disinfection for Aseptic and Support Areas*, which provides the disinfectants used and contact time for facility and equipment as presented in the following table:

The SOP also describes the cleaning method and frequency of cleaning for the facility and equipment as summarized below:

[(b)(4)]

[(b)(4)]

c. The validation of the cleaning/disinfection of the re-usable equipment at Isolagen including -----(b)(4)-----

Isolagen states that they did not perform validation studies for cleaning the (b)(4), -----(b)(4)----- . The sponsor commits to generating cleaning validation protocols for each piece of equipment to ensure the method is effective in reducing bioburden levels. The protocols will start with a baseline environmental monitoring test to determine bioburden load -----(b)(4)-----, followed by the cleaning regimen, then a post-cleaning environmental monitoring test session. The protocol will be conducted under EX-PRT-128, *Cleaning Validation for Routine Manufacturing Equipment*.

d. The clean hold time and dirty hold time for the equipment, if applicable.

Isolagen states that they have not validated clean and dirty hold times for reusable equipment. MBRs instruct the Operator to disinfect the -----(b)(4)----- prior to ---

----- (b)(4) -----

Reviewer’s comments: Isolagen states that no equipment is left in a “dirty state”, however they did not provide references or documentation to support cleaning of the --(b)(4)--- after use, or cleaning the ---(b)(4)--- before and after use. It is not clear whether an –(b)(4)-- is cleaned between lots or just on a routine monthly basis.

e. The routine cleaning/disinfection protocol for the equipment.

----- (b)(4) -----

- ----- (b)(4) -----

----- (b)(4) -----

- ----- (b)(4) -----

----- (b)(4) -----

- ----- (b)(4) -----

Reviewer’s comments: Responses are acceptable for **Q7 b & e**. However, for **Q7 a, c & d**, Isolagen needs to provide the protocol and data of the cleaning validation studies. During the PLI, the firm was cited (483 observation # 5) for the lack of cleaning validations. The cleaning validation studies will be reviewed in the 483 response memo. *Note: This issue is closed from a review standpoint.*

8. You state that the Sterility Assurance Level (SAL) for the --(b)(4)-- 2 ml cryogenic vial is (b)(4). In general, the SAL is 10^{-6} . Please justify your statement.

----- (b)(4) -----

----- (b)(4) -----
-----:

• ----- (b)(4) -----

• ----- (b)(4) -----

• ----- (b)(4) -----

• ----- (b)(4) -----

Reviewer's comments: Response is acceptable.

9. You state that the cryovials exceeded the limit of ----- (b)(4) ----- in the initial and confirmatory testing. You speculate that "the moderately (b)(4) result for ----- (b)(4) ----- than the USP specified limit) may be due in part or in full to the printing on the exterior surface of the vial which was exposed to the extracting medium ". You conclude that the --(b)(4)-- 2mL cryovial is a suitable container closure for your product. Please provide data and documentation to support this conclusion.

----- (b)(4) -----

Reviewer's comments: Isolagen did not conduct the study, and did not provide a timeline for submitting the data. This issue is included in the CR letter-ready comments.

10. The ----- (b)(4) ----- testing method is used to demonstrate the effectiveness of the container closure system to maintain integrity and prevent contamination.

Please provide clarification/additional information to address the following:

a. Please provide data to support the sensitivity of the ---(b)(4)---s method used. What were the positive/negative controls?

Isolagen states they the studies performed by ----- (b)(4) ----- did not demonstrate the sensitivity of the ---(b)(4)--- test. They commit to doing additional

container closure integrity testing using a -----(b)(4)-----
----- with appropriate controls.

b. Have you performed the ---(b)(4)----- test following freezing/thawing of the container closure (simulate freezing of the cryovial)? Please provide your rationale and supporting data.

Isolagen states that vials were not subjected to cryogenic freezing prior to performing the ---(b)(4)----- test. Vials were stored at 2-8°C to simulate Drug Product - Injection shipment conditions in order to test the final container closure system for the process. They add that the additional -----(b)(4)----- test studies will be conducted with vials previously frozen in the vapor phase of liquid nitrogen.

c. Have you performed ---(b)(4)---- at 2-8°C to simulate the storage conditions of the Drug Product-Injection? Please provide your rationale and supporting data.

Isolagen references EX-GTR-117, *Final Container Closure Testing* report, which describes that prior to performing the dye ingress test the cryovials were stored for 48 hours at 2-8°C. This report is provided in Appendix 14 of amendment 125348/0.20 and is submitted in the BLA – module 3, volume 6, section 3.2.S.6, appendix 4.

Isolagen adds that additional microbial ingress test studies will be conducted using vials stored at 2-8°C for 48 hours prior to testing.

Reviewer’s comments: Isolagen proposed new -----(b)(4)----- studies to test the container closure integrity, but they have not provided any data to support the CCIT. This issue is included in the CR letter-ready comments.

11. The data provided in Drug Product Shipping Validation EX-GTR-121 (Section 3.2.P.3.5) show that excursions outside a/the prospectively defined acceptance criteria were observed during this study. -----(b)(4)----- were within the release specification/or cell count and viability 48 hours post-shipping, whereas the -----(b)(4)----- failed to meet the specification/or cell count.

a. You state that testing data sheets, executed batch records and clinical trial reports for lot -----(b)(4)----- were reviewed and no previous atypical result had been identified. We note that lot ---(b)(4)----- was also used in the previous stability study (EX-GTR-116) and was also found to be below the release specification after 48 hours----- (b)(4)----- . Please comment.

Isolagen states that they used this lot for shipping under EX-PRT-116, and EX-PRT-121, and in both cases the lot was below specification for cell count. The firm confirms that review of the manufacturing data for lot -----(b)(4)----- showed no "atypical" results during manufacturing. Drug Substance - Cryovial release testing is within the acceptance limits, and is similar to other patient lots.

Isolagen concludes that the 48 hour results from the studies reported in EX-GTR116 and EX-GTR-121 seem to indicate that this particular patient lot may be more sensitive to a 48 hour hold than other patient lots tested.

Reviewer's comments: Additional shipping studies are needed to assure the reliability and consistency of the shipping method. Isolagen is planning to conduct another shipping study under protocol EX-PRT-130.

This issue is included in the CR letter-ready comments.
