

# CMC REVIEW

**BLA 125348**

## **Division of Cell and Gene Therapies, Office of Cellular, Tissue, and Gene Therapies**

**Reviewed by:**     **J. Terrig Thomas, Ph.D.** \_\_\_\_\_  
                              **Donald Fink, Ph.D.**        \_\_\_\_\_

**Signature**

**Date**

**Concurred by:**

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## EXECUTIVE SUMMARY

**Recommendation:** Based on our review of Isolagen Technologies (now Fibrocell Science) BLA for Isolagen Therapy (now azficel-T), we have determined that a number of product manufacturing and control items need to be resolved before assurance of safety is reached. Some questions remain regarding the validation of aseptic processing and the Quality Systems to investigate and close out deviations. In addition, although the product is manufactured to a high standard there remain outstanding issues related to product stability and quality. These critical review issues are raised in the executive summary and discussed in more detail in the product review. They have been included as letter comments at the end of this executive summary.

**Product Overview:** Isolagen Therapy (IT) is an autologous cell therapy product for improvement of moderate to severe nasolabial fold wrinkles in adults for up to six months. The active ingredient is autologous cultured fibroblasts. The fibroblasts are cultured, using standard methodologies, from three 3-mm punch biopsies (dermal and epidermal layers) taken from a patient's post-auricular area. Fibroblasts, due to their proliferative nature, expand more rapidly in culture than the other cell types present, such as keratinocytes. Fibroblasts represent more than 98% of the final product. Following *in vitro* expansion, the fibroblasts are harvested, quality control tests are performed, and the cell suspension is cryopreserved in vials at a defined cell concentration. When required for clinical use, a dose of cells is thawed, washed, formulated to  $1.0\text{--}2.0 \times 10^7$  cells/ml and shipped to the clinical site at 2-8°C by overnight delivery. The cells are injected intradermally in three separate doses given four to six weeks apart.

The mechanism of action of IT has not been demonstrated. However, the Applicant performs testing of each lot to determine that the product consists of viable fibroblasts that produce collagen. The potency of IT is determined by the combination of cell count, viability, identity as fibroblasts and collagen content. The rationale for the choice of these characteristics for IT potency is based on the premise that fibroblast survival and collagen biosynthesis following injection of IT are likely to be important factors for improvement of nasolabial fold wrinkles.

**Review findings:** One of the key elements in reviewing the CMC manufacturing data is to determine whether the manufacturer is able to produce a safe and consistent product such that, with reasonable assurance, the product administered in the phase III trials will be equivalent to that delivered under licensure. As an autologous cellular product IT is inherently variable from patient to patient and lot to lot. Despite this variability, the Applicant has shown that the release specifications for the IT product have been consistent across the two phase III studies (IT-R-005 and IT-R-006). Consequently, as the manufacturing procedure is relatively straightforward, there is assurance that the Applicant can continue to manufacture the product to the standards outlined in the BLA. However, in the absence of supportive product development data, it is not known whether IT non-responders receive a non-potent product, or are incapable of significantly responding to IT.

For the product validation studies, the weakest support is for stability and product shipping. Studies conducted to validate the shipping container and the stability of the cells throughout the proposed 48 hour shelf life were not sufficiently robust. Further studies are requested to provide more support for IT stability during shipping. In addition, specific deficiencies cited in the form 483 Pre-License Inspection report have not been resolved.

## Isolagen Product CR Comments

1. Outstanding issues identified during pre-license inspection of your manufacturing facility conducted August 31- September 4, 2009 and detailed in form 483 have yet to be addressed.
2. The data provided from the shipping validation studies EX-PRT-116 and EX-PRT-121 failed to support your proposed designation of 48 hour drug product stability under the current conditions of shipment. Data from additional validation studies are required to demonstrate that temperatures can stay within the specified ranges and that the product remains stable for 48 hours. These data should be obtained from studies under actual shipping conditions and include potential extremes of temperature that may be encountered during shipment in summer and winter months. The collagen content assay should also be included in evaluation of product stability in your shipping validation studies.
3. The validation data submitted for the collagen assay conducted as part of the final drug product potency assessment does not support your conclusion that the assay is suitable for its intended purpose. The analytical test method for measurement of collagen content (ATM-004) consists of a -----(b)(4)-----  
----- You have designated the assay as a "limit test", with an acceptance criterion of ----- (b)(4)-----, and examined only limit of detection and specificity in your validation protocol. Due to the characteristics of this assay and its use as part of the potency measurement of the drug product, we expect validation of the parameters of linearity, range, accuracy, robustness and precision as outlined in ICH Q2(R1) "Validation of Analytical Procedures: Test and Methodology." Data from an additional validation protocol conducted in accordance with ICH Q2(R1) are required.
4. Please provide the data obtained from proposed study EX-PRT-124 "*Qualification and Comparability of* -----(b)(4)-----.
5. During the Pre-License Inspection on 9-1-09 it was noted that morphologic assessment is performed by -----  
----- (b)(4) -----  
-----  
-----  
-----
6. During the preparation of Bulk Drug Substance, the SOPs require -----  
----- (b)(4) ----- However, during the pre-license inspection dated August 31- September 4, 2009, it was noted from the master batch records that the time ----- (b)(4) ----- is not specified. -----  
----- (b)(4) ----- is not recommended and may result in poor outcomes with respect to product quality. Please change the relevant SOPs to avoid this possibility.
7. We note that during the pivotal studies, lots exhibiting deviations in the time limits for culture at various steps in the process were allowed to proceed on a case-by-case basis. Clear criteria for time limits that would result in lot termination at each critical manufacturing step

need to be established prior to commercialization. Similarly, clear criteria need to be established regarding the use of ----- (b)(4) ---.

8. Under CTD section 3.2.S.2.2 it was noted that a time limit has not yet been established for the ----- (b)(4) -----  
-----  
-----  
A study (EX-PRT-129) has been proposed to address this issue. We recommend that a similar study is also conducted to establish a time limit for the ----- (b)(4) -----  
-----  
-----  
Please submit to the BLA the results of the studies and the hold times that you establish for these steps.
9. 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.
10. The container closure failed ----- (b)(4) ----- in the initial and confirmatory testing. The cryovials tested ----- (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.
11. At the CTGTAC meeting held on 10-9-09 it was noted that morphological criteria cannot definitively prove whether or not a cell is transformed. Although no safety concerns were raised during the clinical trials regarding events linked to cellular transformation, we recommend you develop a test method for assessment of cell transformation for use during manufacture of the commercial product.
12. We request that the post-approval stability protocol for the Drug Substance outlined in section 3.2.S.7.2 be modified to include the --(b)(4)-- assay. Although the --(b)(4)-- assay was not performed on the Drug Substance for manufacture of clinical lots, data obtained on --- (b)(4) --- production from lots on stability would provide valuable information for the assessment of stability.
13. The current identity/purity assay for fibroblasts and keratinocytes is based on independent analyses for the ----- (b)(4) -----  
-----  
-----  
This method does not provide information on cells not detected by --- (b)(4) -----. Please address this concern, either by providing data to adequately demonstrate the quantity and type of cells ----- (b)(4) -----  
-----  
-----  
or by adding a quality control test.
14. -----  
-----  
----- (b)(4) -----  
-----  
-----  
-----  
-----

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## 3.2.S DRUG SUBSTANCE

### 3.2.S.1 General Information

#### 3.2.S.1.1 NOMENCLATURE

**United States Adopted Name (USAN)**

azficel-T

**International Non-proprietary Name (INN)**

Not applicable

**Company names, Trivial Name, or codes used to identify the product in the application**

Isolagen Therapy™

#### 3.2.S.1.2 STRUCTURE

Isolagen Therapy™ has no defined chemical structure, because it is an autologous cellular product.

#### 3.2.S.1.3 GENERAL PROPERTIES

Isolagen Therapy™ (IT) is an autologous cell therapy product composed of a suspension of autologous fibroblasts cultured from a biopsy of each individual's own skin. Skin tissue (dermal and epidermal layers) is biopsied from a patient's post-auricular area and shipped via next day delivery to Isolagen's manufacturing facility in Exton, PA. Fibroblasts are isolated from the tissue by enzymatic digestion --- (b)(4) --- and expanded to a quantity sufficient for re-injection into the patient's treatment area. For the Isolagen clinical studies IT-R-005 and IT-R-006, the treatment area was specific to the nasolabial fold regions.

Isolagen Therapy™ (IT) Drug Substance-Cryovial consists of fibroblasts that are harvested from the ----- (b)(4) -----, formulated to the desired cell concentration and cryopreserved in cryovials. Drug Substance cryovial is stored in a cryopreservation medium consisting of ----- (b)(4) ----- dimethyl sulfoxide (DMSO) to a target of ----- (b)(4) ----- . The cryopreserved Drug Substance is stored in the vapor phase of a controlled rate liquid nitrogen freezer.

### 3.2.S.2 MANUFACTURER

Manufacturer Name	Address	Contact
Isolagen Technologies, Inc.	405 Eagleview Blvd Exton, PA 19341	John Maslowski, M.S., CQA Vice President of Operations



		Phone: 484-713-6032 Fax: 484-713-6001 Email: jmaslowski@isolagen.com
--	--	--

Manufacture of the Bulk Drug Substance-Cryovial is performed at Isolagen's Exton, PA facility (Tissue Establishment Registration Number 3005836954).

The Exton facility is a ----- (b)(4)--- square foot, aseptic autologous cellular therapy manufacturing building. All cellular processing is done inside a -----(b)(4)----- cleanroom and all operations follow cGMP (see CTD section 3.2.A.1.)

All commercial in-process and release testing of the -----(b)(4)----- and Bulk Drug Substance-Cryovial is performed at Isolagen's Exton facility, with the exception of mycoplasma testing which is be outsourced to -----(b)(4)----- (Facility Establishment Registration Number --- (b)(4)----. Analytical procedures are presented in CTD Section 3.2.S.4.2.

### **3.2.S.2.2 MANUFACTURING PROCESS AND PROCESS CONTROLS**

Isolagen Therapy™ (IT) is an autologous cell therapy product composed of a suspension of autologous fibroblasts cultured from a biopsy of each individual's own skin. The drug substance consists of fibroblasts that are harvested from the -----(b)(4)-----, formulated to the desired cell concentration and cryopreserved in cryovials (Drug Substance-Cryovial).

The current manufacturing process is outlined in the flow diagram in Figure 1 (SEE BELOW).

**35 pages redacted due to (b)(4)**

**Letter comment:** The current identity/purity assay for fibroblasts and keratinocytes is based on independent analyses for the -----(b)(4)----- . This method does not provide information on cells not detected by ----(b)(4)----. Please address this concern, either by providing data to adequately demonstrate the quantity and type of cells not -----(b)(4)----- or by adding a quality control test.

### Cell Count and Viability

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

### **3.2.S.3.2 IMPURITIES**

This section describes known and potential impurities in the Isolagen Therapy™ (IT) Drug Substance-Cryovial lots derived from the manufacturing process.

#### Summary of Potential and Expected Process-Related Impurities

##### Endotoxin

	IT-R-005 and IT-R-006
Total number of tests	(b)(4)
Below Detection Limit	
Above Detection Limit	
Minimum Detected Level	
Maximum Detected Level	

- Endotoxin levels have been measured as a QC release test for all Drug Substance-Cryovial lots manufactured in compliance with -----(b)(4)-----
- The detection limit for endotoxin is ----(b)(4)---- for assays using the --(b)(4)-----  
------(b)(4)-----.
- Of the (b)(4) Drug Substance-Cryovial lots tested for endotoxin during Isolagen Phase 3 clinical studies IT-R-005 and IT-R-006, -----(b)(4)----- contained endotoxin levels below the level of detection for the assay.
- The level of detectable endotoxin in (b)(4) lots ranged from ----(b)(4)----, well below the lot release specification of -(b)(4)---

#### **INFORMATION REQUEST SUBMITTED TO THE SPONSOR AS A LETTER COMMENT ON 8-11-09**

11. In the Impurities section (3.2.S.3.2) for Drug Substance characterization, in Phase 3 clinical studies IT-R-005 and IT-R-006, (b)(4) lots were analyzed for identity, whereas ---(b)(4)----lots were tested for endotoxin. Please comment.

**Response (Amendment #19 10-30-09):**

We reviewed Section 3.2.S.3.2 and identified an error in Table 2. In the Phase 3 studies IT-R-005 and IT-R-006, (b)(4) lots were tested for endotoxin and gave values below the detection limit of the assay, and (b)(4) lots gave values above the detection limit, for a total of (b)(4) lots tested for endotoxin. (b)(4) of the lots tested for endotoxin -----(b)(4)----- were not analyzed for identity. (b)(4) of these were found to be Out-of-Specification (OOS) for Cell Count and Viability prior to identity analysis and identity testing was discontinued. Lot ----(b)(4)---- was not tested for identity due to a procedural error. For convenience of review, the original and a revised Table 2 for Section 3.2.S.3.2 are provided below, with changes indicated in italics.

**Table 5. Original Table 2. Endotoxin Detection in Drug Substance-Cryovial Lots**

	IT-R-005 and IT-R-006
Total number of tests	(b)(4)
Below Detection Limit	
Above Detection Limit	
Minimum Detected Level	
Maximum Detected Level	

**Table 6. Revised Table 2. Endotoxin Detection in Drug Substance-Cryovial Lots**

	IT-R-005 and IT-R-006
Total number of tests	(b)(4)
Below Detection Limit	
Above Detection Limit	
Minimum Detected Level	
Maximum Detected Level	

**Reviewer's comment:** This clarifies the differences in the numbers of lots analyzed for identity and endotoxin levels.

**Potential Impurities Derived from the Manufacturing Process**

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

## Residual Gentamicin and Amphotericin B

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

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

- \_\_\_\_\_(b)(4)\_\_\_\_\_
- \_\_\_\_\_(b)(4)\_\_\_\_\_
- \_\_\_\_\_(b)(4)\_\_\_\_\_
- \_\_\_\_\_(b)(4)\_\_\_\_\_
- \_\_\_\_\_(b)(4)\_\_\_\_\_

### Residual Fetal Bovine Serum (FBS)

- As FBS is primarily a concern for Drug Product-Injection, please see section **3.2.A.2**

## Product-Related Impurities

The IT Drug Substance-Cryovial does not include any product-related impurities.

**Reviewer's comment:** While not a safety concern, as the viability specification is (b)(4)- there is the potential impurity of non-viable cells.

### 3.2.S.4 CONTROL OF DRUG SUBSTANCE

A description of the specifications used for the release of Drug Substance and justification thereof is provided

### 3.2.S.4.1 SPECIFICATIONS

The specifications for the Drug Substance-Cryovial are listed below. All tests are conducted at the Exton facility, except mycoplasma testing, which is outsourced to -----(b)(4)-----  
-----  
A complete description of the analytical methods is included in section  
3.2.S.4.2.

Gram staining is not included as the results of the 14-day sterility test will be available prior to patient administration.

Test	Method (ATM)	Tentative Specifications
<b>Safety Testing:</b>		
Sterility	Sterility Testing (ATM-003)	Negative
Mycoplasma	FDA Points to Consider (1993) [REDACTED]	[REDACTED]
<b>Purity and Identity Testing:</b>		
Purity	Purity Assay Using the [REDACTED] System (ATM-006)	[REDACTED]
Endotoxin	Endotoxin Testing (ATM-002)	[REDACTED]
<b>Potency and Viability Testing:</b>		
Cell Count	[REDACTED]	[REDACTED]
Cell Viability	[REDACTED]	[REDACTED]

### 3.2.S.4.2 ANALYTICAL PROCEDURES

*See review under section 3.2.P.5.2*

### 3.2.S.4.3 VALIDATION OF ANALYTICAL PROCEDURES

*See Review under section 3.2.P.5.3*

### 3.2.S.4.4 BATCH ANALYSES

#### **Batch Description**

- As each patient's cells grown for treatment are a unique lot number, an extensive lot history is present for IT batches.
- QC in-process and release testing for IT Drug Substance batches used in clinical Trials IT-R-005 and IT-R-006 are provided.
- Example Drug Substance Certificates of Analysis from IT-R-005 and IT-R-006 are also provided.

- Test results indicated as Not Applicable (NA) are either because the testing was discontinued due to a specification failure or due to termination of a subject from the study -----(b)(4)----- and testing was not completed.
- Batch records for lots used in stability and process validation studies are provided

### **3.2.S.4.5 JUSTIFICATION OF SPECIFICATIONS**

#### **Sterility**

- The specification for sterility is based on conformation to the ----(b)(4)---- monograph.

#### **Mycoplasma Testing**

- Mycoplasma testing is performed as a Drug Substance-Cryovial release test, but is conducted as an effective in-process test -----(b)(4)-----.
- The release specification of a negative result is based on conformation to the May 1993 FDA Points to Consider recommended procedure.
- All bulk ----(b)(4)---- for use in clinical investigations tested -----(b)(4)-----.

#### **Adventitious Viral Agent Testing**

- Testing for adventitious viral agents is not performed as a release test as IT is an autologous cellular therapy and does not require such testing in accordance with 21 CFR 1271.90(a)(1).

#### **Endotoxin Testing**

- Drug Substance-Cryovial lots are tested for endotoxin in accordance with ---(b)(4)---.
- The specification of ----(b)(4)--- is based on (b)(4) of the maximum allowable level for an injectable product in ----(b)(4)---.
- Of the (b)(4) lots tested during clinical studies IT-R-005 and IT-R-006, -----(b)(4)----- were below the level of detection.
- Of the (b)(4) lots that were above the level of detection, the average value was ---(b)(4)--- and the maximum was ----(b)(4)---.
- As a result of the data collected to date, an alert limit of ----(b)(4)---- has been established and an investigation as to the cause will be performed on lots exceeding the alert limit.

#### **Purity Testing**

- Drug Substance-Cryovial lots are tested for fibroblast cell purity based on -----(b)(4)----- (see 3.2.S.3.1).
- The specification of (b)(4) purity is based on data reported from the Phase 3 clinical studies IT-R-005 and IT-R-006.
- Of the (b)(4) lots assayed, (b)(4) met the specification, with an average purity of --(b)(4)----.
- -----(b)(4)----- detectable keratinocytes and of the (b)(4) lots that did, the maximum did not --- (b)(4)---.

### **Cell Count**

- (b)(4) cell count is used as a test for potency for IT Drug Substance lots.
- The specification of -----(b)(4)----- is based on data reported from the Phase 3 clinical studies IT-R-005 and IT-R-006.
- Of the (b)(4) lots that were assayed for cell count -----(b)(4)----- met the specification.
- Lots not meeting the specification were either adjusted to the proposed specification or were not used to prepare Drug Product-Injection lots.

### **Viability**

- Cell viability is also used as a test for potency for IT Drug Substance lots.
- The specification of -(b)(4)- is based on data reported from the Phase 3 clinical studies IT-R-005 and IT-R-006.
- Of the (b)(4) lots that were assayed for cell count -----(b)(4)----- met the specification.
- The (b)(4) lots which had a viability of (b)(4) were not used to prepare Drug Product-Injection.

### **Collagen Content**

- Collagen content is not determined on the Drug Substance. Justification for the collagen specification is provided under section 3.2.P.5.2.

## **3.2.S.5 REFERENCE STANDARDS OR MATERIALS**

- No drug-specific reference standards are used for testing Drug Substance-Cryovial, as each lot is obtained from a unique population of fibroblasts derived from a single patient.

## **3.2.S.6 CONTAINER CLOSURE SYSTEM**

*Review to be provided by DMPQ*

## **3.2.S.7 STABILITY**

Isolagen has conducted stability studies on Bulk Drug Substance-Cryovial lots filled in the commercial container closure system and stored in the vapor phase of liquid nitrogen using stability protocol EX-PRT-014. Three stability studies were conducted at the Exton facility:

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The assays conducted for the stability studies were the -----(b)(4)-----  
----- lot were used for testing at -----(b)(4)----- used for sterility testing  
and (b)(4) for other analytical testing. For time points where sterility was not tested, -----(b)(4)-----  
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## 3.2.P DRUG PRODUCT

### 3.2.P.1 DESCRIPTION AND COMPOSITION OF DRUG PRODUCT

The Isolagen Therapy™ (IT) Drug Product-Injection consists of a suspension of autologous fibroblasts formulated in DMEM without phenol red. IT is supplied as two 2 ml vials each containing 1.2 ml of Drug Product at  $1.0 - 2.0 \times 10^7$  cells/ml.

#### CONTAINER CLOSURE SYSTEM

*Review to be conducted by DMPQ*

#### COMPOSITION OF DRUG PRODUCT

##### Drug Substance

The source of fibroblast cells are thawed vials of Drug Substance-Cryovial that are washed in -----(b)(4)----- of ----- (b)(4)----- of DMEM without phenol red, before final suspension at the target cell concentration. A detailed description of Drug Product-Injection preparation is provided in section 3.2.P.3.3.

##### Excipients

The only excipient present in Drug Product-Injection is DMEM without phenol red. A detailed description is provided in section 3.2.P.4 and cross reference authorization letters to the Drug Master Files from -----(b)(4)----- are provided. Although DMEM is -----(b)(4)-----, it has been used in an approved product, Carticel™ (BLA 96-0372). It presents low risk to patients as evidenced by its use in all the clinical trials conducted by Isolagen under IND for various indications. (b)(4) is used to wash the cells from Drug Substance-Cryovial and residuals may be present in Drug Product-Injection in small quantities.

#### Formulation Development

- In the first clinical study under IND (IT-R-001),  $0.5 - 3.0 \times 10^7$  cells/ml were formulated.
- In study IT-R-002, the formulation was narrowed to  $1.0 - 3.0 \times 10^7$  cells/ml
- In all subsequent studies, the formulation was further narrowed to  $1.0 - 2.0 \times 10^7$  cells/ml.

### 3.2.P.2.3 MANUFACTURING PROCESS DEVELOPMENT

An overview of Drug Product preparation, optimization of critical process steps and determination of growth potential following Drug Product-Injection Storage is provided.

#### Summary

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**3.2.P.2.4 CONTAINER CLOSURE SYSTEM**

*Review to be provided by DMPQ*

**3.2.P.2.6 COMPATIBILITY**

This section addresses the compatibility of the drug product with reconstitution diluent and dosage devices.

**Diluents**

- Isolagen Therapy™ (IT) is supplied ready for intradermal injection and should not be diluted prior to administration as the efficacy of IT at concentrations below the supplied dose formulation has not been evaluated.

**Dosage Devices**

- Cells are provided as a suspension in a 2.0 ml screw-cap polypropylene container
- The cell suspension is withdrawn from the container using a small unit syringe fitted with a detachable needle
- While a 29 or 30-gauge needle is required for intradermal injection, a larger bore 21-gauge needle may be used to aid in withdrawing the product from the container.
- The compatibility of using a 29 or 30-gauge needle for administration of the IT Drug Product-Injection was addressed in stability study EX-GTR-086, *Stability Study for Shipment and Room Temperature Handling of Drug Product-Injection*.
- -----(b)(4)----- after holding at room temperature --(b)(4)-----
- -----(b)(4)-----  
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**3.2.P.3 MANUFACTURE**

- Manufacture of the Bulk Drug Product-Injection is performed at Isolagen’s Exton, PA facility (Tissue Establishment Registration Number 3005836954).
- All commercial in-process and release testing of the Drug Product-Injection will be performed by Isolagen’s Exton facility.

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### 3.2.P.3.4 CONTROL OF CRITICAL STEPS AND INTERMEDIATES

The production of Isolagen Therapy™ (IT) Drug Product-Injection from Drug Substance-Cryovial is a short process consisting of a number of ----- (b)(4) ----- a final resuspension in DMEM (without phenol red) with no hold steps. Therefore, no intermediates exist for the Drug Product-Injection manufacturing process.

#### *Critical Process Controls for Drug Product*

- Drug Product-Injection release specifications are listed in the table below. No additional parameters have been identified as critical process controls for Drug Product-Injection

manufacturing.

Parameter (ATM)	Test	Specification
Endotoxin (ATM-002)		
Gram Stain (ATM-001)		Negative
Sterility <sup>1</sup> (ATM-003)		Negative
Cell Count (ATM-005)		1.0 to 2.0 x 10 <sup>7</sup> cells/mL
Cell Viability (ATM-005)		
Collagen (ATM-004)		

<sup>1</sup>Final results of Sterility testing of Drug Product – Injection are not received until after product release.

**Reviewer's Comment:** During the PLI on 9-2-09 the sponsor provided information on the Product Accountability Controls used for labeling and tracking the source material and the product throughout the manufacturing process. While tracking and labeling procedures are described in various SOPs throughout the manufacturing process, we suggested compiling a Master Tracking/Labeling SOP, incorporating each of these SOPs as a point of reference.

### 3.2.P.3.5 PROCESS VALIDATION/EVALUATION

#### Validation of Sterilization Procedures

Review of Media Fill validation to verify that all aseptic procedures and containers yield sterile materials with no contamination to be completed by DMPQ

#### Validation of Shipping Procedures

##### *Drug Product Shipping Procedure*

- Following Drug Product-Injection sterile fill, a shipper container is assembled according to SOP-180 (*Packaging Procedure for Final Product*) and described in CTD sections **3.2.P.2.4 and 3.2.P.7.**
- While the shipping container is being assembled, the Drug Product-Injection vials to be shipped are stored at -----(b)(4)----- prior to shipping.
- Verification of labeling and sample identification is performed by QA.

##### Drug Product Shipping Validation Study: EX-PRT-116 (*Shipping Validation of Isolagen Drug Product-Injection*)

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**Letter comment:** The data provided from the shipping validation studies EX-PRT-116 and EX-PRT-121 failed to support your proposed designation of 48 hour drug product stability under the current conditions of shipment. Data from additional validation studies are required to demonstrate that temperatures can stay within the specified ranges and that the product remains stable for 48 hours. These data should be obtained from studies under actual shipping conditions and include potential extremes of temperature that may be encountered during shipment in summer and winter months. The collagen assay should also be included in evaluation of product stability in your shipping validation studies.

**Reviewer’s Comment:** *With regard to commercial lot shipping failures, and positive sterility results obtained post-release; a clinical letter comment was included requesting instructions and activities thereof be incorporated into the Clinical Support Center Policies and Procedures.*

**d.** Under section 3.2.P.2.3, a stability limit of 48 hours from Drug Product preparation was set, in which Drug Product-Injection is shipped overnight at  $5 \pm 3^{\circ}\text{C}$  to be used within 48 hours. The above study demonstrates that your process is not validated for the set specification. Additional studies are needed to validate that the shipping container and conditions are suitable for the transport of the Drug-Product-Injection. As you suggested, we also recommend additional post-licensure shipping stability studies be performed to reinforce the validation of cell count/viability post-shipment. In addition to cell count and viability, these studies should also include the collagen content functional assay. Please submit a proposed protocol for a post-marketing stability study to the BLA for review.

***Response (Amendment #19 10-30-09):***

*Fibrocell is currently drafting the post-marketing stability study protocol to examine shipping stability in detail between 24 and 48 hours and will submit the proposed protocol to the BLA for review. This protocol, EX-PRT-130, 48 Hour Drug Product Shipping Stability Study, will be executed using research and development lots manufactured post-marketing.*

**Actions Taken for Drug Product Shipping Failures**

- The clinical site will verify the condition of the Drug Product shipper upon arrival.
- If any shipper damage is observed, the clinical site will contact the Clinical Administrator as described in SOP-702, *Clinical Notification*, and the Clinical Administrator will contact Isolagen Quality Assurance.

**INFORMATION REQUEST SUBMITTED TO THE SPONSOR AS A LETTER  
COMMENT ON 8-11-09**

16. Under Actions Taken for Drug Product Shipping Failures (Section 1.2.4) you state that the clinical site will verify the condition of the Drug Product shipper upon arrival and if any shipper damage is observed, the clinical site will contact the Clinical Administrator as described in SOP-702, *Clinical Notification*, and the Clinical Administrator will contact Fibrocell Quality Assurance. To ensure patient safety, please provide information on the course of action to be taken in the event of a shipping failure.

***Response (Amendment #19 10-30-09):***

*In the event of a shipping failure such as; a delay in receipt of the shipper beyond the validated shipping time, damage that compromises the integrity of the shipper, or inappropriate temperature upon receipt, the clinical site will be instructed to:*

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**Reviewer's comment:** *Please see letter comment in response to question 15c above*

## **PROCESS VALIDATION RESULTS**

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### **INFORMATION REQUEST SUBMITTED TO THE SPONSOR AS A LETTER COMMENT ON 8-11-09**

17. The final 14 day sterility results for Process Validation study EX-PRT-110 (Section 1.3) were not available at the time of submission of the BLA. Please submit the final sterility results to the BLA.

#### ***Response (Amendment #19 10-30-09):***

*Final sterility test results for Drug Substance – Cryovial and Drug Product – Injection are presented in EX-GTR-110 (refer to Page 42, Table 31 and Page 43, Table 32, respectively, in appendix 9 of BLA section 3.2.P.3.5). The final report and raw data for the sterility tests are provided in Appendix 3 of this submission. All results were negative in the sterility test.*

**Reviewer's comment:** *This is acceptable*

### 3.2.P.4 CONTROL OF EXCIPIENTS

**REVIEWER ASSESSMENT:** Fibrocell (Isolagen) has provided information related to the control of excipients used in the manufacturing process. Suppliers of these materials are identified, details about the formulation of the materials is provided as are samples of Certificates of Analyses supplied with received materials describing product testing performed by the manufacturer/supplier.

Information described in this section of the BLA submission highlights specifications established for receipt of materials and their qualification for use in the manufacture of LaViv and provides for specification justification. Additionally, Fibrocell (Isolagen) has described testing performed on excipients once they are received at the Exton, PA manufacturing facility, provided analytical test methods developed for the purpose of excipient testing and submitted validations of the analytical test methods demonstrating their suitability for performing the testing outlined.

#### *Dulbecco's Modified Eagle Medium (DMEM) / ----(b)(4)-----*

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### 3.2.P.5 CONTROL OF DRUG PRODUCT

#### 3.2.P.5.1 Drug Product Specifications

The specifications for the final Drug Product-Injection outlined below are additional to the Drug Substance specifications outlined in section 3.2.S.4.1

Test	Method (ATM)	Tentative Specifications
Safety Testing:		
Sterility	(b)(4)	Negative
Gram Stain		Negative
Purity Testing:		
Endotoxin		(b)(4)
Potency and Viability Testing:		
Cell Count		1.0 – 2.0 x 10 <sup>7</sup> cells/mL
Cell Viability		(b)(4)
Collagen Content		

### Analytical Test Methods/Procedures and Validation (BLA Sections 3.2.S.4.2, 3.2.S.4.3, 3.2.P.5.2 and 3.2.P.5.3)

#### OVERALL REVIEWER ASSESSMENT:

Isolagen (Fibrocell) has developed analytical assays and designed/executed validation protocols that demonstrate the suitability of these assays for performing release testing of LaViv™ Bulk Drug Substance - Cryovial and Drug Product – Injection. Validation protocols have been designed in accordance with principles and recommendations contained in various regulatory documents including CFRs, ICH, FDA Guidance, and USP. The assays developed are appropriate for the characteristics of the product under investigation. Execution of validation experiments met system suitability and results satisfied predetermined acceptance criteria. Statistical analyses of assay parameters assessed under validation protocols are relevant in circumstances when the assay parameter evaluated supports a quantitative assessment. The assay validation packages described in the BLA submission for (1) Sterility ---(b)(4)--- and Gram stain), (2) Purity (endotoxin), (3) Cell Count/Viability, (4) Purity/Identity and (5) Mycoplasma are acceptable. The analytical assay Collagen Content, part of the potency matrix assessment, requires additional validation.

#### SUMMARY – ANALYTICAL TEST METHODS / VALIDATION:

The information contained in **Sections 3.2.S.4 and 3.2.P.5** of the CTD-BLA Submission describes analytical test methods developed for the purpose of establishing the quality of the Drug Substance (Cryovial) and Drug Product (Injected). The following tests are common to

both the Bulk Drug Substance – Cryovial and Drug Product - Injected: (1) Sterility, (2) Endotoxin, (3) Cell Count, (4) Cell Viability, and (5) Identity – Phenotypic Analysis: Fibroblasts/Keratinocytes.

At the time of Bulk Substance -----(b)(4)----- testing for mycoplasma contamination is performed. For Drug Product (Injected) a Gram stain is conducted -----(b)(4)----- . In addition, a test for collagen expression is conducted as a -----(b)(4)----- potency assay (b)(4).

The assays developed to perform quality assessment and release of Drug Substance / Drug are based on guidance documents, compendial methods, and in-house assay development. An analytical test method has been developed for each assay. Validation protocols have been designed and executed for each analytical test method in order to demonstrate the suitability of the individual assays for their intended purpose. Where appropriate, validation approaches were used to demonstrate assay accuracy, precision/repeatability, specificity, detection limit, quantitation limit, linearity, range and robustness.

The information provided in the BLA submission demonstrates the analytical test methods developed are suitable for evaluating product quality in association with performing final product release testing.

#### **ANALYTICAL TEST METHODS / VALIDATION OF TEST METHODS– --(b)(4) DRUG SUBSTANCE - CRYOVIALS**

- **Sterility:** ---(b)(4)---- / ATM-003 (3.2.S.4.2: **Appendix 1**) – Test performed at Fibrocell (Isolagen) according to Fibrocell (Isolagen) Analytical Test Method ATM-003 in compliance with ---(b)(4)---, Sterility Tests. Direct inoculation of culture medium for detection of microbial contaminants.
- **Endotoxin:** ---(b)(4)--- / ATM-002 (3.2.S.4.2: **Appendix 2**) – -----(b)(4)----- Assay; -----(b)(4)-----.
- **Cell Count / Viability:** ATM-005 and ATM-008 (3.2.S.4.2: **Appendix 1 and Appendix 5**)
- **Purity/Identity:** ATM-006 (3.2.S.4.2: **Appendix 6**)

**Table: Proposed Commercial Release Specifications for Drug Substance - Cryovial**

<b>DRUG SUBSTANCE - CRYOVIAL</b>		<b>Test Method</b>	<b>In-Process Release Specifications</b>
Sterility		----(b)(4)----	No Growth Detected
Endotoxin		----- (b)(4) -----	---(b)(4)---
Cell Count		Flow Cytometry --- (b)(4) ---	----(b)(4)-----
Cell Viability		Flow Cytometry --- (b)(4) ---	(b)(4)
Identity	Fibroblasts	----- (b)(4) -----	----- (b)(4) -----
	Keratinocytes	----- (b)(4) -----	-----

**Table: Proposed Commercial Release Specifications for Drug Product – Injection**

TEST	ANALYTICAL TEST METHOD	PROSPECTIVE SPECIFICATIONS
<b>Safety Testing:</b>		
Sterility	------(b)(4)-----	Negative / No Growth Detected
Gram Stain	------(b)(4)-----	Negative / No Growth Detected
<b>Purity Testing:</b>		
Endotoxin	------(b)(4)-----	------(b)(4)-----
<b>Potency/Viability Testing:</b>		
Cell Count	------(b)(4)-----	1.0 – 2.0- x 10 <sup>7</sup> cells/mL
Cell Viability	------(b)(4)-----	(b)(4)
Collagen Content	------(b)(4)----- -----	------(b)(4)-----

- All testing is performed by Fibrocell (Isolagen) at the Exton, PA facility.

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**Table: Completed Method Validation/Verification Protocols – Drug Substance and Drug Product**

RELEASE TEST ASSAY	ANALYTICAL TEST METHOD	VALIDATION PROTOCOL	CTD SECTION WITH VALIDATION DATA
Sterility	ATM-003	SOP-333 / EX-PRT-045 v00	3.2.S.4.3 and 3.2.P.5.3
Gram Stain (Drug Product Only)	ATM-001	EX-PRT-87-v00	3.2.P.5.3
Endotoxin	ATM-002	EX-PRT-056 v01 / EX-PRT-057 v01	3.2.S.4.3 and 3.2.P.5.3
Mycoplasma	------(b)(4)-----: -----	EX-PRT-027 v00	3.2.S.4.3
Cell Count / Viability	ATM-005	EX-PRT-079 v00	3.2.S.4.3
Collagen Content	ATM-004	EX-PRT-118 v00 and v01	3.2.P.5.3



## Sterility

### ***STERILITY ANALYTICAL TEST METHOD – (BLA Sections 3.2.S.4.2 and 3.2.P.5.2 – APPENDICES 1: ATM-003/STERILITY TESTING/ Effective Date 16-Oct-2008)***

Purpose: outline the method for detection of microbial contaminants by direct testing sterility method. Procedure complies with -----(b)(4)----- Sterility Tests.

Scope: procedure applies to required sterility testing for -----(b)(4)-----, (b) Bulk Drug Substance – Cryovial, and (c) Drug Product – Injection Samples

#### Definitions:

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Sampling: Sample for each lot of Drug Substance-Cryovial produced analyzed for sterility.

#### Procedure:

- GENERAL:
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**Reviewer Note:** The frequency -----(b)(4)----- is performed was discussed with the sponsor during pre-license inspection of the manufacturing facility in Exton, PA (08-31-09 to 09-04-09). The sponsor stated that due to the physical properties of the -----(b)(4)----- sample (cloudy appearance prior to inoculation), it is not uncommon for a -----(b)(4)----- step to be performed.

- SPECIFICATIONS AND INTERPRETATION OF RESULTS
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**VALIDATION OF STERILITY ANALYTICAL TEST METHOD ATM-003:** Performed in -----(b)(4)----- with Standard Operating Procedure SOP-333: BACTERIOSTASIS AND FUNGISTASIS ACTIVITY TEST under Validation Protocol EX-PRT-045: ISOLATION OF FIBROBLASTS USING -----(B)(4)-----3.2.P.5.3 – Appendix 7)

- Sterility testing performed as part of Validation Protocol EX-PRT-045 (Release Testing of Bulk Drug Substance - Cryovial) using Analytical Technical Method (ATM) – 003 (described above) for both Drug Substance – Cryovial and Drug Product–Injection.

- SOP-333: Bacteriostasis and Fungistasis (3.2.S.4.3 – Appendix 7 and 3.2.P.5.3 – Appendix 6) developed/executed as verification of compendial ---(b)(4)---- specified in -----(b)(4)-----, Sterility: purpose is to demonstrate lack of interfering substances within Drug Substance – Cryovial with respect to sterility test results.

***SOP-333: Bacteriostasis and Fungistasis Activity Test***

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## Gram Stain

### ***GRAM STAIN ANALYTICAL TEST METHOD – (BLA Section 3.2.P.5.2 – APPENDIX 2:ATM-001 / GRAM STAIN / Effective Date 20-Oct-2008)***

PURPOSE: Describes method for performing Gram stain test using both the --(b)(4)--- and --(b)(4)---- Method when testing -----(b)(4)-----and Drug Product – Injection samples.

SCOPE: Procedure applies to Gram stain test which is used as a microbial contamination detection tool for performing release testing of Drug Product – Injection. Test Method provides instructions/interpretations to be used for both testing approaches.

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## Endotoxin

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***Reviewer note concerning mycoplasma testing validation/ verification:***

*Pre-License Inspection – Exton, PA Facility (August 30 – September 4, 2009): During the facility pre-license inspection and review of relevant SOP and test result documents it was noted that the analytical test method for mycoplasma release testing of the Drug Substance – Cryopreserved (Protocol (b)(4): Mycoplasmal Screening for ----- (b)(4) ----- has been developed and executed by ----- (b)(4) -----), the laboratory contracted by Fibrocell (Isolagen) to perform mycoplasma release testing. In contrast, the analytical test method validation protocol EX-PRT-027: Mycoplasma Detection with ----- (b)(4) ----- Guidelines provided in the BLA submission has been developed and executed by ----- (b)(4) -----). The validation protocol only addresses the issues of assay interference ----- (b)(4) ----- testing) but does not reflect execution of the --- (b)(4) --- mycoplasma test protocol for sample analysis. This arrangement involving ----- (b)(4) ----- of the mycoplasma test verification is not acceptable for demonstrating test method validation compliance. Fibrocell (Isolagen) was informed of this deficiency during the pre-license inspection.*

*On Friday, September 04, 2009 at 9:51 AM EDT Fibrocell (Isolagen) received a facsimile transmission dated 03-Sep-2009 from -----*

*----- (b)(4) -----  
stating the company has not conducted a validation of its Points to Consider Mycoplasma analytical test method. The company has initiated execution of a historical validation study that has a target completion date of 01-January-2010.*

*483 Observation (04-September-2009): Currently, mycoplasma release testing of the bulk Drug Substance – Cryovial is performed by the contract company ----- (b)(4) -----  
-----; however, the mycoplasma test method Protocol (b)(4) has not been validated/verified (assay performed in accordance with FDA Points To Consider: Characterization of Cell Lines used to Produce Biologicals [1993]) by the contract laboratory.*

*Fibrocell (Isolagen) Response to Form FDA 483 – Inspectional Observations (17-September-2009): Fibrocell (Isolagen) acknowledged that ----- (b)(4) ----- is the contract laboratory specified for mycoplasma testing as reported in the BLA submission whereas -----  
----- (b)(4) ----- used to execute the ---- (b)(4) ----- validation studying  
----- (b)(4) ----- . Reported that ---- (b)(4) ---- has validated mycoplasma Points to Consider Testing in accordance with FDA’s Recommended Procedures for Detection of Mycoplasma Contamination in Biological Products Produced in Cell Substrates, Attachment of Points to consider in the Characterization of Cell Lines Used to Produce Biologicals (May 1993). Documentation was not provided substantiating this statement.*

*Fibrocell (Isolagen) referenced the September 3, 2009 memo received from ---- (b)(4) ---- indicating retrospective validation testing of contract laboratory’s Mycoplasma PTC (b)(4) -----  
----- will be completed with a target date of January 1, 2010. Upon completion, -- (b)(4) ---- will provide Fibrocell (Isolagen) with a completed validation report which will be forwarded upon review to FDA as a BLA amendment submission. In the interim, Fibrocell (Isolagen) will not use --- (b)(4) --- to perform release testing of commercial product until an appropriate validation report has been submitted to the BLA. Fibrocell (Isolagen) will use --- (b)(4) --- for commercial product Mycoplasma PTC testing. Once the validation report received from --- (b)(4) --- is submitted to the BLA and reviewed by FDA, Fibrocell (Isolagen) intends to resume using --- (b)(4) -- as the contract laboratory for performing mycoplasma testing.*

*Telecon with Fibrocell (Isolagen) Regarding Responses to 483 Inspectional Observations – Mycoplasma Testing Validation (23-October-2009): During a telephone discussion between CBER Staff (BLA review of CMC and Facilities Information) and Fibrocell (Isolagen) representatives, sponsor was informed that the following information describing mycoplasma testing performed by --- (b)(4) ---- needs to be submitted to the BLA: (1) copy of the test method protocol, (2) description of experience and training required by technical staff performing mycoplasma testing, and (3) a summary of test results for PTC mycoplasma testing performed by --- (b)(4) ---- over previous -- (b)(4) --- period demonstrating consistency and reliability of testing performed. This will serve to verify that ---- (b)(4) ---- is executing mycoplasma testing as described in the May 1993 FDA Points to Consider Document.*

*BLA Amendment S/N 023 Received 25-November-2009: Amendment submitted by Fibrocell in response to information requested during 23-October-2009 telecon with BLA-CMC Review Team. The information provided in BLA Amendment 023 has been supplied by -----(b)(4)----- and includes the following:*

- 1. Copy of Protocol #30055: Mycoplasma Detection – “Points to Consider”. Designed to demonstrate test article consisting of cells is----- (b)(4)----- according to FDA “Points to Consider” criteria.*
- 2. Synopsis of Test Training requirements expected of technicians in the Mycoplasma Testing Laboratory that includes instruction concerning GLP and cGMP practices. Training considered complete for the mycoplasma test procedure upon evidence the trainee is able to correctly execute the procedure and/or give a correct verbal explanation of the procedure. Specific training for mycoplasma test procedure includes familiarity with SOP for Aseptic Techniques and Practices.*
- 3. Summary of Recent Mycoplasma Testing performed by -----(b)(4)-----*
  - Total of (b)(4)- “Points to Consider” tests executed in accordance with protocol #30055 between July 1, 2009 and October 30, 2009.*
  - All (b)(4) of completed assays met assay suitability/validity requirements including expected results for positive and negative Mycoplasma control samples.*
  - All (b)(4)-tests considered valid, none judged to be invalid.*

**REVIEWER ASSESSMENT:** The information supplied in BLA Amendment 024 in conjunction with details provided in the BLA submission (EX-PRT-027: Mycoplasma Detection with -----(b)(4)-----, 3.2.S.4.3 – Appendix 3) verify contract laboratory ----(b)(4)----- is capable of valid execution of the Points to Consider Mycoplasma Detection assay. The combination of the mycoplasma detection test with the assay for -----(b)(4)----- indicate this testing is suitable for evaluation of Bulk Drug Substance for evidence of mycoplasma contamination.

### **Cell count/Viability**

***CELL COUNT/VIABILITY ANALYTICAL TEST METHOD – (BLA Section 3.2.S.4.2 – APPENDIX 4: ATM-005: CELL COUNT AND VIABILITY WITH THE -----(b)(4)-----***  
***-----***

**PURPOSE:** Analytical Test Method (ATM) outlines procedure for determining cell count/cell viability of Bulk Drug Substance – ----(b)(4)---- Drug Substance – Cryovials and Drug Product – Injection using the -----(b)(4)-----

### **PRINCIPLE:**

- Cell count and viability analytical method executed using the -----(b)(4)----- is conducted at Isolagen in accordance with ATM-005 (Appendix 4).*
- Method used to determine the cell count/viability of ----(b)(4)--- and Drug Substance-Cryovial lots.*

**3 pages redacted due to (b)(4)**



- Acceptable alternative method for determining cell count/viability on samples that are too --(b)(4)-- or otherwise cannot be assayed using ATM-005: *Cell Count and Viability with* -----(b)(4)-----

**Reviewer Note:** During the pre-license facility inspection performed 30-August 2009 through 04-September-2009, the sponsor was asked to describe conditions that contribute to samples which are ---(b)(4)--- or otherwise cannot be assayed using the -----(b)(4)-----.

**Sponsor Response:** Only rarely, if ever, are samples received in QC following -----(b)(4)----- for analysis using the -----(b)(4)----- . The most likely contributor would be the result of--(b)(4)-- reaching a ---(b)(4)----- than expected for the period of time in --(b)(4)-- based on SOPs that govern the manufacturing process.

The circumstance of “or otherwise cannot be assayed” arises from the fact that, until recently, the QC laboratory had only one qualified -----(b)(4)----- that was being used to perform cell counting, viability, and identity testing. On those occasions when the -----(b)(4)----- was being used to perform other time-dependent assays or was unavailable due to performance suitability check as the result of an unplanned shutdown, then cell counting and testing could be performed using the -----(b)(4)----- .

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### **Purity/Identity**

***PURITY/IDENTITY ANALYTICAL TEST METHOD – (BLA SECTION 3.2.S.4.2 – APPENDIX 6 /ATM-006: PURITY ASSAY USING THE ----- (b)(4)-----***

Analytical test method for purity/identity is performed by the Quality Control Unit at Fibrocell (Isolagen) according to ATM-006: *Purity Assay using the -----(b)(4)-----*. The assay is conducted to determine -----(b)(4)----- of fibroblasts (derived from the dermis) and keratinocytes (principal cell type in the epidermis) that are present in the Drug Substance –

Cryovial which is formulated to produce Final Drug Product – Injection without further processing. This test is not repeated after final formulation prior to final product release. Fibrocell refers to the test method as a purity assay with respect to determining cell types present in the cellular product, but it serves as an identity assay confirming fibroblasts comprise the majority proportion of the cell contents in the final formulated product. Assay features include:

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#### **3.2.P.5.4     Batch Analyses**

A complete list of all QC results for (b)(4) IT Drug Product-Injection batches used in the IT-R-005 and IT-R-006 clinical trials are provided, whether or not they were injected.

#### **3.2.P.5.5     CHARACTERIZATION OF IMPURITIES**

The impurities described for Drug Substance in section **3.2.S.3.2** will be -----  
----- (b)(4) ----- of Drug Product-Injection. In the  
final Drug Product-Injection the impurities likely to be present are:

### **Keratinocytes**

- Non-fibroblast cells derived from the tissue biopsy are potential impurities in the Drug Product.
- Keratinocytes are the major potential other cell type that may be present.
- A release specification of  $\geq 98\%$  fibroblasts and -(b)(4)- other cell types has been set.
- Analytical QC release data from the Isolagen Phase 3 clinical studies IT-R-005 and IT-R-006 determined the average purity of the Drug Substance-Cryovial to be --(b)(4)-- fibroblasts.
- In these studies (b)(4) of lots --(b)(4)-- had no detectable keratinocytes, Of the (b)(4) lots that did contain measurable quantities of keratinocytes, the maximum did not --- (b)(4)-----.

### **Fetal Bovine Serum (FBS) and Dimethyl Sulfoxide (DMSO)**

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### Endotoxin

- Residual endotoxin is tested according to ATM-002 in compliance with ----(b)(4)-----  
-----.
- The Drug Product-Injection release specification is ---(b)(4)----
- All lots produced for clinical studies IT-R-005 and IT-R-006 were well below this specification and (b)(4) of the lots were below the level of detection -----(b)(4)----- .
- No endotoxin test result exceeded ---(b)(4)----.

### CONTAINER CLOSURE SYSTEM LEACHABLES

*To be reviewed by DMPQ*

### 3.2.P.5.6 JUSTIFICATION OF SPECIFICATIONS

#### Current Release Specifications for Drug Product-Injection

Test	Analytical Method (ATM)	Tentative Specifications
Safety Testing:		
Sterility	Sterility Testing (ATM-003)	Negative
Gram Stain	Gram Stain (ATM-001)	Negative
Purity Testing:		
Endotoxin	Endotoxin Testing (ATM-002)	(b)(4)
Potency and Viability Testing:		
Cell Count	(b)(4)	1.0-2.0 x10 <sup>7</sup> cells/mL
Cell Collagen Content	Collagen Assay (ATM-004)	(b)(4)
Cell Viability	(b)(4)	
(b)(4)		

### Summarized Drug Product-Injection Release Data

The table below includes a statistical summary of the quantitative release data for the (b)(4) lots produced for clinical studies IT-R-005 and IT-R-006.

[(b)(4)]

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### **Specifications for Safety Testing**

#### Sterility and Gram Stain Testing

- Sterility testing is in accordance with ---(b)(4)--- using ATM-003 and Gram staining using ATM-001. **ANALYTICAL TEST METHODS/PROCEDURES AND VALIDATION SECTION (3.2.P.5.2 and 3.2.P.5.3)**

### **Specifications for Purity Testing**

- Drug Product-Injection lots are tested for endotoxin in accordance with --(b)(4)--- using ATM-002.
- The proposed specification of --(b)(4)--- is based on (b)(4) of the maximum allowable level for an injectable product in ---(b)(4)---.
- All Drug product lots under IT-R-005 and IT-R-006 met the specification
- Of the (b)(4) lots manufactured, (b)(4) lots were below the level of detection for the assay
- Of the (b)(4) lots that were above the level of detection, the maximum value was (b)(4)
- The majority of lots with detectable levels of endotoxin occurred in June 2007 and was later determined to be due to a higher than expected level of endotoxin in a raw material.



- Following discussion with FDA at the pre-BLA meeting, an alert limit of ---(b)(4)----- has been established for endotoxin and an investigation of cause will be performed for all lots exceeding the alert limit.

## Potency and Viability Testing

### Potency

The Isolagen Therapy potency assay is a combination of cell count, cell viability and collagen production

- For release of the (b)(4) IT Drug Product-Injection lots for pivotal clinical studies IT-R-005 and IT-R-006, cell count and cell viability were considered adequate to assess the potency of Drug Product.
- Upon agreement with FDA, a cell collagen content assay was developed and will be included as one of the Drug Product potency release tests for the commercial product.

### *Cell Collagen Content Assay*

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[(b)(4)]

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***Response (Amendment #19 10-30-09):***

*The final Drug Product – Injection cell count specification is based on the dose of azficel-T used during U.S. commercial distribution prior to regulation, and subsequent clinical trials under IND. The safety of azficel-T for doses of 0.5, 1.0 and  $2.0 \times 10^7$  cells/ml were demonstrated in IND study IT-R-001. In studies IT-R-003A/B, IT-R-005 and IT-R-006 the cell count specification for release was  $1.0\text{-}2.0 \times 10^7$  cells/ml.*

*Whereas there may be some variability in the final DP-I cell count after overnight shipping to the clinical site, this variability did not appear to substantially impact the findings of safety and efficacy in pivotal studies. Fibrocell agrees to collect data regarding the clinical effects of azficel-T post-approval and will consider modification to the DP-I cell count specification should tightening this specification be appropriate.*

**Reviewer's comment:** *This is response is adequate*

*Viability*

- ▶ Drug Product-Injection lots are tested for viability as described in ATM-005.
- ▶ The specification of -(b)(4)- cell viability is based on viability requirements for clinical studies.
- ▶ In clinical studies IT-R-005 and IT-R-006 the average cell viability was ---(b)(4)----- and (b)(4) lots (b)(4) were not released because the viability specification was not met.

[(b)(4)]

**INFORMATION REQUEST SUBMITTED TO THE SPONSOR AS A  
LETTER COMMENT ON 8-11-09**

18. In the Justification of Specifications section (3.2.P.5.6) under Potency, the number of Drug Product preparations appears to vary from (b)(4) (identity testing) to (b)(4) (potency testing). Please explain this discrepancy.

***Response (Amendment #19 10-30-09):***

*For clarity, section 3.2.P.5.6, titled Potency describes cell count (N=(b)(4), viability (N=(b)(4) collagen content (N=(b)(4) and endotoxin testing (N=(b)(4) results and trends.*

*Of the (b)(4) lots tested for cell count, (b)(4) of the lots failed to meet the cell count or viability specification. According to Fibrocell release testing procedures for DP-I, no further release testing is conducted on lots that do not meet the cell count and viability specifications. As such, these (b)(4) lots were not tested for endotoxin.*

*One additional lot of the noted (b)(4) lot difference, did pass cell count specifications but was discontinued due to an environmental monitoring (EM) excursion (high particle count) during DP-I manufacturing. Thus, this lot was also discontinued from further release testing (i.e., endotoxin testing).*

**Reviewer's comment:** *This explanation is acceptable*

### **3.2.P.6 REFERENCE STANDARDS OR MATERIALS**

- No product specific reference standards are used for testing Drug Product-Injection, as each Drug Product lot is obtained from a unique population of fibroblasts derived from each patient.

### **3.2.P.7 CONTAINER CLOSURE SYSTEM**

*Review to be conducted by DMPQ*

### **3.2.P.8 STABILITY**

#### **3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment**

Isolagen proposes to place (b)(4) lots of Bulk Drug Substance-Cryovial and Drug Product-Injection, manufactured according to the commercial process, on routine stability testing per year.

- Post-approval stability studies for Bulk Drug Substance-Cryovial are described in CTD section **3.2.S.7.2**.
- The Drug Product-Injection lots will be prepared, stored 2-8°C for 0, 24 and 48 hours and subjected to release testing.
- All lots will be required to pass release specifications as outlined below.

[ (b)(4) ]

**Reviewer's comment:** *As indicated in the letter comment associated with the response to stability studies in section 3.2.P.3.5 we request that collagen content be included in future stability studies*

### 3.2.P.8.3 Formal Stability Study Data

Three formal stability studies were conducted on Drug Product-Injection

#### Stability Study for Drug Product-Injection (EX-PRT-013)

The table below provides the overall stability study design and specifications to be met for lots following storage at 2-8°C in a laboratory setting

Test Method	Specification	Time Point (Days)
Gram Stain	----- (b)(4) -----	----- (b)(4) -----
Endotoxin	----- (b)(4) -----	(b)(4)
Sterility	----- (b)(4) -----	-(b)(4)--
Cell Count	----- (b)(4) -----	----- (b)(4) -----
Cell Viability	--(b)(4)--	----- (b)(4) -----

- Three lots were tested – ----- (b)(4) -----.
- All three lots passed the acceptance criteria for endotoxin, Gram Stain and Sterility at all the time points measured.
- For cell count:
  - ▶ ----- (b)(4) -- passed specification -----
  - ▶ ----- (b)(4) -- passed specification -----
  - ▶ ----- (b)(4) -- passed specification -----
- For cell viability, all three lots maintained specification through (b)(4)
- The results demonstrate that the final Drug Product-Injection lots maintained all release specifications for up to 48 hours when stored at 2-8°C.

#### Stability Study for Shipment and Room Temperature Handling of Drug Product-Injection (EX-PRT-086)

This stability study was designed to test the stability of the Drug Product-Injection stored at 2-8°C for 24 hours to mimic shipping conditions and then held at 20-25°C for --- (b)(4) ----- to mimic how the product might be handled in the clinic. In addition, after storage at (b)(4) -----, the Drug Product-Injection lots were drawn up in a 21-gauge needle and expelled through a 30-gauge needle to mimic patient injection.

The table below provides the overall stability study design and specifications to be tested.

Test Method	Specification	Time Point (Days)
Gram Stain	----- (b)(4) -----	(b)(4)
Endotoxin	----- (b)(4) -----	(b)(4)
Sterility	----- (b)(4) -----	(b)(4)
Cell Count	----- (b)(4) -----	---- (b)(4) -----
Cell Viability	-- (b)(4) --	---- (b)(4) -----

- Three lots were tested, ----- (b)(4) ----- and the results demonstrate that the final Drug Product-Injection lots maintained all release specifications for all the time points tested.

*Process Validation Lots Final Product Stability (EX-PRT-112)*

This laboratory stability study was part of EX-PRT-110, *Isolagen Standardized Manufacturing Process Validation Protocol* in which three Drug Product -Injection lots were stored at 2-8°C and subjected to stability testing for up to 48 hours.

The table below provides the overall stability study design and specifications to be tested.

Test Method	Specification	Time Point (Days)
Gram Stain	----- (b)(4) -----	-- (b)(4) --
Endotoxin	----- (b)(4) -----	(b)(4)
Sterility	██████████	(b)(4)
Collagen	----- (b)(4) -----	-- (b)(4) --
Cell Count	----- (b)(4) -----	-- (b)(4) --
Cell Viability	- (b)(4) -	-- (b)(4) --

- All three lots tested ----- (b)(4) ----- passed all release specifications through -- (b)(4) --.
- It is noteworthy that, in all three lots tested, the collagen content measurement -- (b)(4) --- with storage time, indicating that the cells were both viable and biologically active.

[ (b)(4) ]

## **Shipping Stress Studies**

EX-PRT-086 - *Stability Study for Shipment and Room Temperature Handling of Drug Product-Injection*

- This study demonstrated Drug Product-Injection stability under storage and handling conditions encountered at the clinical sites, and is described above.

EX-PRT-121 - *Twenty-Four Hour Shipping Validation of Isolagen Drug Product-Injection*

- This study is described in CTD section **3.2.P.3.5**.

***Reviewer's comment: The stability studies EX-PRT-013, EX-PRT-086 and EX-PRT-112 conducted under various laboratory conditions demonstrated product stability for up to 48 hours following final product preparation. However stability studies EX-PRT-116 and EX-PRT-121 conducted under actual shipping conditions failed to validate the 48 hour stability time period. See letter comment to sponsor in section 3.2.P.3.5.***

## **3.2.A APPENDICES**

### **3.2.A.2 Adventitious Agents Safety Evaluation**

Isolagen Therapy™ (IT) is an autologous cell product. Consequently, no donor screening or testing of cells or tissues is required [21 CFR 1271.90(a)(1)]. No human-derived reagents are used during manufacture of IT.

-(b)(4)- reagents are used during manufacture of IT that are potential sources of animal-derived adventitious agents are.

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- ▶ Informed consent form was revised to notify subjects participating in the clinical trial of the potential risks associated with treatment derived from bovine sourced materials.
- ▶ Isolagen informed the investigators participating in IT-R-005/006 and IT-R-007 as to the potential risks of using bovine sourced materials in the production of IT.
- ▶ Isolagen notified the IRBs for these studies through submission of a revised informed consent form and Investigator's Brochure.
- ▶ Isolagen indicated to FDA that manufacturing for the IT-R-005/006 and IT-R-007 studies was already in process at the time Isolagen learned of the issue. No further biopsies are expected for these studies.

**INFORMATION REQUEST SUBMITTED TO THE SPONSOR AS A LETTER  
COMMENT ON 8-11-09**

20. -----(b)(4)----- was used for preparation of clinical lots for IT-R-005 and IT-R-006 (Section 3.2.A.2.1).

*a.* What will be the source of -----(b)(4)----- to be used for the commercial process?

*b.* If not -----(b)(4)-----, how will this -----(b)(4)---- be qualified for use?

***Response (Amendment #19 10-30-09):***

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#### Summary of Risk Assessment for Animal Origin Raw Materials

The source, supplier testing, and additional testing by Isolagen provides a high degree of assurance of a low risk of contamination of IT by adventitious viral, bacterial, mycoplasma, fungal or transmissible spongiform encephalopathy agents from animal origin raw materials and materials produced using animal origin raw materials.

#### **3.2.A.2.3      Control of the Production Process**

The risk of IT contamination with adventitious agents is also minimized by the conditions of manufacture.

- All cell culture manipulations are carried out in -----(b)(4)-----  
----- within an -----(b)(4)----- environmental background using aseptic precautions.
- Drug Product-Injection is tested for endotoxin and microbial contamination by Gram Stain and sterility testing.
- Drug Substance-Cryovial is tested for endotoxin, mycoplasma and sterility.

### **3.2.R      REGIONAL INFORMATION**

#### **3.2.R.1.S      Executed Batch Records (EBRs) for Drug Substance**

As agreed with the FDA during the Type B pre-BLA meeting, three Drug Substance-Cryovial lot EBRs are provided. All manufacturing was conducted using the process for commercialization described in CTD section 3.2.S.2.2.

- EBRs for lots -----(b)(4)----- are provided.
- The process order for use of Master Batch Records (MBRs) for manufacture of Drug Substance-Cryovial is listed below:

**2 page redacted due to (b)(4)**

### **Additional information from CTGTAC meeting held on 10-9-09**

Following the CTGTAC held on 10-0-09 and a subsequent consult with panel member Steven Dubinett, M.D. on 10-23-09 the following recommendations were made:

CTGTAC Concern	Current Testing	Proposals	Comments	Consideration	Recommendation
Tumorigenicity	<p>Morphological analysis – rejection of lots exhibiting abnormal growth characteristics</p> <p>No lots rejected during pivotal trials and no serious adverse events reported</p>	<ul style="list-style-type: none"> <li>Karyotype analysis - Neoplastic cells often have a karyotype deviant from the normal diploid state</li> <li>Histopathology screening for abnormal cells using portion of larger biopsies</li> <li>Histopathology of final product</li> <li>Analysis of tumor suppressor proteins</li> <li>Transformation testing by ----- --(b)(4)----- -----</li> </ul>	<p><u>Pro</u>: use to reject lots exhibiting abnormal aneuploidy/polyploidy <u>Con</u>: in an aging population there will be a number of aneuploidy/polyploidy events not related to transformation</p> <p><u>Pro</u>: reject if abnormal/carcinoma cells observed <u>Cons</u>: portion of biopsy examined may not represent cultured cells.</p> <p><u>Pro</u>: reject if carcinoma cells observed <u>Con</u>: limited test sample size will only detect carcinoma cells if they are abundant</p> <p><u>Pro</u>: rejection of lots showing aberrant profiles of tumor suppressor proteins <u>Con</u>: basis for setting the criteria is unclear</p> <p><u>Pro</u>: assay in principle is simple, and can be quantitative – reject lots producing colonies <u>Con</u>: will only detect fast growing tumor cells. No direct correlation with <i>in vivo</i> tumor formation. Validation may be difficult</p>	<p>Information obtained may be difficult to interpret without clear data in literature on which to base an acceptance criterion</p> <p>Biopsy does not represent the final product. Clinicians already instructed on obtaining biopsy from normal skin.</p> <p>Would improve safety profile if sufficient cells could be detected</p> <p>Information difficult to interpret</p> <p>Would add to the existing safety profile if validated. However, no clear data on correlation with <i>in vivo</i> tumor formation.</p>	<p>Post-marketing evaluation of ----- ----- (b)(4) ----- ----- ----- ----- Data should also be collected from suitable ----- --- (b)(4) ----- -----</p> <p>Post-marketing study on a subset of --(b)(4)----- ----- The results to be submitted for review.</p> <p>No recommendation</p>
Product Characterization	<p>Identity Testing using mutually ---(b)(4)----- ----- fibroblasts and keratinocytes. Release test: ≥ 98% fibroblasts (b)(4) keratinocytes</p> <p>(b)(4) collagen assay on final product Release Spec: (b)(4)----- ----- ---</p>	<ul style="list-style-type: none"> <li>Identification of “other” cell types in the final product, in particular Mast cells, which could promote keloid formation</li> <li>Determine the amount and ratio of collagen types I/III produced <i>in vitro</i></li> </ul>	<p><u>Pro</u>: markers available for a more comprehensive analysis of any ‘other’ cell types present in the final product <u>Con</u>: minor cell types may be below the level of detection. Literature suggests other cell types will not be present after culture.</p> <p><u>Pro</u>: Info on types of collagen produced <i>in vitro</i>. <u>Con</u>: Collagen production <i>in vitro</i> may not correlate to clinical outcome. Mechanism of action unknown, so interpretation of data unclear</p>	<p>Identification of other cell types is possible and would provide a more comprehensive evaluation, but unlikely to add to the safety profile of the final product</p> <p>Data could be collected on --(b)(4)--- lots to see if there is a correlation with clinical outcome</p>	