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LABORATORIES

FOR THE MEDICAL INDUSTRY WORLDWIDE

P.O. Box 140 • 1001 Proctor Drive • Elkhorn, WI 53121-0140
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GLOBAL HEALTH SOLUTIONS

CurX Antimicrobial Wound Dressing
Lot No.: 15F10E3

Cytotoxicity Evaluation
Agar Overlay/L929 Mouse Fibroblast
[ANSI/AAMI/ISO 10993-5:2009(R)2014]
(GLP)

March 14, 2016

JN16B0973

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SECTION 1

TEST PROTOCOL

GLP0002*

JN16B0973

GENEVA LABORATORIES
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GLP PROTOCOL

Cytotoxicity Evaluation
(Agar Overlay/L929 Mouse Fibroblast)
[ANSI/AAMI/ISO 10993-5:2009/(R)2014]

SPONSOR: Global Health Solutions P.O. No. CLS0005
P.O. Box 133 /1360 Redmond Circle, NW
Rome, GA 30162

TEST ARTICLE: CurX Antimicrobial Wound Dressing

LOT/ID: 15F10E3

Signing of this protocol constitutes your approval of the procedure outlined on the following pages.

STUDY DIRECTOR: _____ STUDY INITIATION DATE: 2-22-2016
Vince Lazzaroni
Study Director/Microbiology
Geneva Laboratories, Inc.

SPONSOR: Bradley Burnam DATE: 2/24/2016
Global Health Solutions

16-014

GENEVA LABORATORIES, INC.

PROTOCOL FOR CYTOTOXICITY EVALUATION
(Agar Overlay/L929 Mouse Fibroblast)
[ANSI/AAMI/ISO 10993-5:2009(R)2014]
Title 21 CFR Part 58
Good Laboratory Practice for a Nonclinical Laboratory Study

§ 58.120 PROTOCOL

1). TITLE

Cytotoxicity Evaluation (Agar Overlay/L929 Mouse Fibroblast)
Geneva Laboratories Procedure No.: CC1002*, Add. ISO-01*

2). PURPOSE

To assess biological reactivity of mammalian cells (grown in culture) to the agar-diffusable elements from either component materials or finished medical products.

NOTE: The selection of the Agar Diffusion (Cytotoxicity) test method is justified based on its historical application for evaluating this class of product (ref. sponsor).

3). IDENTIFICATION OF

	Name	CAS/Code (Lot No.)
Test Article:	CurX Antimicrobial Wound Dressing	15F10E3
Positive Control:	Amber Latex Tubing	P/N 62996-473 VWR Scientific
Negative Control:	HDPE Sheet Stock	P/N 46009; U.S. Plastic Corp.
Monolayer Negative Control:	Cell Culture Dishes w/Agar Overlay Medium added	Current Stock (varies by test date)

*Denotes Current Revision

4). SPONSOR

Global Health Solutions
P.O. Box 133/1360 Redmond Circle, NW
Rome, GA 30162
ATTN: Mr. Brad Burnam

5). TEST FACILITY

Geneva Laboratories, Inc.
Proctor Drive at McKenzie Lane
P.O. Box 140
Elkhorn, WI 53121-0140

6). TEST SYSTEM

L929 Mouse Fibroblast Cell (serial subculture), purchased from either Diagnostic Hybrids or Sigma Aldrich (as qualified).

7). TEST SYSTEM IDENTIFICATION

Monolayers of L929 Mouse Fibroblast Cells, cultured in test dishes, are labelled with the appropriate cell line, cell passage number and date of passage.

8). DESCRIPTION OF EXPERIMENTAL DESIGN

Testing will be conducted in accordance with established Geneva Laboratories procedures. A summary of the methods is found below.

A. Preparation of Sample/Test Materials

1. Test samples (test article/controls) will each be sized to have no less than 100 mm^2 of contact surface and will provide coverage of approximately 10% of the test dish.

2. The manipulations of the test article and controls will be performed aseptically. While a sanitization step may be employed for non-sterile samples, it is generally unnecessary due to the incorporation of an antibiotic/antimycotic in the culture media.

3. The media will be prepared by combining equal parts of 2x Minimum Essential Medium (with 2% Fetal Bovine Serum) and Agar Noble immediately prior to the test exposure.

B. Test Exposure

1. The monolayer test dishes will have been microscopically examined to assure the cell growth is subconfluent (80 ±10% confluent), of normal morphology and free of contamination.

2. The entire volume of media in each of the test dishes will be carefully drawn off (so as not to disturb the cells) and replaced with a suitable volume of tempered, agar-supplemented culture media.

3. After the agar has solidified, triplicate portions of the test article* and controls will be gently positioned on the overlay medium (one sample per dish).

   *NOTE: When the test article has only one face designated for patient-contact, that "side" of the article is to be directed toward the agar.

4. The test dishes, including three (3) each containing the agar medium only (Monolayer Negative Control), will be inverted and incubated.

   NOTE: If a test article is massive in nature (i.e. dense) and/or does not adhere well to the agar, the test dishes may be incubated "upright".

C. Bias is controlled by randomly sampling the various parts of the test article and using equal portions of the various parts when the test article is not homogenous. Also, the test system is homogenous and from one source.

9.) TEST SYSTEM MAINTENANCE

A. Media

1. Prior to the exposure phase, the cell monolayers will have been cultured using MEM with 10% FBS as a growth medium.

2. The volume of media used per test dish will be in accordance with the guidance described in "Basic Laboratory Techniques in Cell Culture", CDC, 1981.

3. After supplanting the growth medium with the Agar Medium preparation, no further supplementation will occur.

B. Environmental Controls

1. The test system (monolayer dishes) will be held at 37 ±1°C for the twenty-four (24) hour minimum observation period.

2. A humidified atmosphere of 5% CO_2 will be maintained throughout to minimize pH effects.

10). TYPE AND FREQUENCY OF TEST MEASUREMENTS

A. At twenty-four (24) + two (2) hours after exposure, the test plates will be microscopically examined for indication of cellular response. A preliminary examination will be conducted prior to staining and fixing the cells.

NOTE: Per ISO test guidelines, a longer exposure interval may be used if desired, up to seventy-two (72) hours.

B. The condition of the cells will be described and graded as per the referenced procedure. A numerical scale ranging from "0" (no response) to "4" (severe response) will be used.

C. A cytological stain (i.e. Neutral Red) will be employed at the final reading to help visualize the response.

D. The use of formalin as a fixative will assure that the integrity of the cells is maintained as the agar is removed from the test dishes.

11). RECORDS TO BE MAINTAINED

All raw data that is the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of that study will be maintained in the Geneva Laboratories archives.

12). PROPOSED STATISTICAL METHODS

None.

13). REVISIONS TO PROTOCOL

All changes in or revisions of an approved protocol and the reasons for the change will be documented, signed and dated by the Study Director and maintained with the protocol.

14). DATA INTERPRETATION

The referenced procedure provides criteria for interpreting the observed responses.

A. Control samples must elicit the normally anticipated results for a valid assay.

1. The negative controls may be no greater than a Grade 0.

2. The positive control response may be no less than a Grade 3.

B. For the test article, the following apply:

1. Grades of 0, 1 (slight) or 2 (mild) indicate the article "meets" the assay acceptance criteria.

2. Grades of 3 (moderate) or 4 (severe) do not meet assay acceptance criteria.

C. Other than the acceptance criteria described above, no statistical methods are used.

SECTION 2

TEST REPORT/STUDY PERSONNEL

GENEVA LABORATORIES
FOR THE MEDICAL INDUSTRY WORLDWIDE
P.O. Box 140 • 1001 Proctor Drive • Elkhorn, WI 53121-0140
Phone: (262) 723-5669 • **Fax:** (262) 723-4015
www.genevalabs.com

OVERLAY-M

REPORT TO: Mr. Bradley Burnam
Global Health Solutions
P.O. Box 133/1360 Redmond Circle, NW
Rome, GA 30162

TEST ARTICLE: CurX Antimicrobial Wound Dressing
Lot No. 15F10E3

P.O. NO.: CLS0005

DATE RECEIVED: 02-12-2016

TEST INITIATION DATE: 03-02-2016 TEST COMPLETION DATE: 03-04-2016

TEST PROCEDURE: Cytotoxicity Test-Agar Overlay/L929 Mouse Fibroblast
Ref. Geneva Laboratories Proc. No. CC1002; Rev. Q
Add. ISO Modification; ISO-01; Rev. C
(GLP)

OBJECTIVE: To assess the biological reactivity of mammalian cells (grown in culture) to the agar-diffusable elements from either componment materials or finished medical products.

CONCLUSION: Under the conditions of this study, the Test Article:

X Meets test acceptance requirements
N/A Does not meet test acceptance requirements

For a detailed description of test methods and findings, see pages 2-6.

ANALYST: _____ DATE: 3.11.2016

ACCEPTED BY: _____ DATE: 03.11.2016
Technical Reviewer

QA SIGNATURE: _____ DATE: 03-11-2016

TEST METHODS:

A. Sample Preparation

The test article and control materials were prepared in accordance with the American National Standard ANSI/AAMI/ISO 10993-5: 2009/(R)2014.

Test Article
Dimensions: 1.1 x 1.1 cm, 1.1 x 1.15 cm, 1.1 x 1.2 cm

Positive Control [Amber Latex Tubing (VWR), Lot W034-070]
Dimensions: 1.0 x 2.55 cm, 1.0 x 2.7 cm, 1.05 x 2.7 cm

Negative Control [HDPE Sheet Stock (US Plastics), Lot 10152013]
Dimensions: 1.15 x 1.2 cm, 1.15 x 1.15 cm, 1.0 x 1.15 cm

B. Cell Preparation

Prior to the exposure phase, the cells were subcultured to achieve a confluency of approximately 80 ±10% at the time of exposure. Each of the test dishes was identified with the cell line, cell passage number and date of passage.

Just prior to exposure, each of the dishes was microscopically examined for possible contamination and to observe if the level of confluency required had been achieved.

Percentage confluency at time of use: 80-90%

Once found to meet the acceptance criteria for use in the test, individual dishes were numbered (in triplicate) to represent the controls and the test articles.

C. Cell Exposure

On the day of testing, the subculture media was carefully removed from each test dish and replaced with a two (2) mL aliquot of the 1:1 overlay medium.

Media Used: 2X MEM w/2% FBS; Lot: 03022016
Agar Noble, Lot: 09172015

After allowing the overlay medium to solidify, a single test article or control specimen was placed in the center of each dish (in contact with the agar surface). Triplicate cultures were prepared for each test article and positive and negative controls.

The test dishes, along with three (3) dishes with overlay medium only (Monolayer Negative Controls), were then placed in the 37°C ±1°C/5% CO_2 incubator to initiate the exposure interval.

Exposure Date: 03-02-2016
Exposure Interval: 24 1/4 hrs.

D. Cellular Staining and Fixation

After completion of the incubation period, the cells were stained with a fresh working Neutral Red Solution to facilitate response grading. The test article and control materials were removed from the dishes at this time.

The stained cells were then "fixed" by the addition of 10% buffered formalin. Following fixation, the agar overlay was removed from each dish.

E. Observations

A preliminary microscopic examination of the cells was made prior to staining and before the control and test specimens were removed from the agar layer.

Following the staining process, the cellular responses were then evaluated microscopically and macroscopically (by examining the dishes against a white surface) and the results recorded.

E. Observations (cont'd)

See Table below for grading guidelines.

Grade(1)	Reactivity	Description of Reactivity Zone(2)
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen(3)
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extends 0.45 to 1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond specimen

NOTE(1): The use of the above Grading Table is contingent on the test article meeting the minimum surface area requirements of ≥100 mm^2. Should samples of smaller dimensions be tested, the reactivity (if any) would be expected to be less and the grading would need to be justified.

NOTE(2): The extent of the Reactivity Zone is the maximum measured distance from the edge of the specimen to the margin of monolayer where degenerated cells are no longer observed. Where described as "under specimen", this maximum measured distance is limited to <0.45 cm beyond the specimen.

NOTE(3): To be interpreted as "slight" reactivity, no more than 50% of the cells under the specimen may exhibit reactivity as rounding and/or lysis.

F. Additional Methods:

Any additional steps required to complete the test that was not described above are indicated here:

N/A

RESULTS: See table below for the cellular responses from the controls and test article.

		Macroscopic Reading (Zone Dimensions)	Microscopic Reading (% Rounded/Lysed)	Grade
Monolayer Negative Control	1	No detectable zone	0% / 0%	0
	2	No detectable zone	0% / 0%	0
	3	No detectable zone	0% / 0%	0
Material Positive Control	1	Clear Zone 3.2 x 3.2cm Greatest distance from specimen 1.5cm	100% / 100%	4
	2	Clear Zone 3.2 x 3.2cm Greatest distance from specimen 1.5cm	100% / 100%	4
	3	Clear Zone 3.2 x 3.2cm Greatest distance from specimen 1.5cm	100% / 100%	4
Material Negative Control	1	No detectable zone	0% / 0%	0
	2	No detectable zone	0% / 0%	0
	3	No detectable zone	0% / 0%	0
Test Article	1	Entire dish lightly stained ≈5% rounded directly under sample	1.5% / 1.5%	1
	2	Entire dish lightly stained ≈5% rounded directly under sample	1.6% / 1.6%	1
	3	Entire dish lightly stained ≈5% rounded directly under sample	1.6% / 1.6%	1

Date Read: 03-04-2016
Read By: Vince Lazzaroni

OBSERVATIONS:

N/A

INTERPRETATION OF RESULTS:

A. Assay controls must meet the acceptance criteria for a valid assay.

   All negative control responses must be no greater than a Grade 0.

   Positive control responses must be no less than a Grade 3.

B. The responses observed from the test articles are interpreted according to the current USP guidance.
   Grades of 0 (None), 1 (Slight), or 2 (Mild) indicate the article meets the assay acceptance requirements.

   Grades of 3 (Moderate) or 4 (Severe) indicate the test article does not meet the assay acceptance requirements.

C. No statistical analyses of the data are being made.

CONCLUSION:

The Grade 1 test response from the sample preparation is considered to be "non-cytotoxic" (i.e. meets ISO test acceptance requirements of no more than Grade 2 reactivity).

GENEVA LABORATORIES, INC.

Microbiology Department
Cytotoxicity Test Personnel

Vince Lazzaroni -- Study Director

Sharon Smith -- Study Director (pro tempore)

E. Jane Lewis -- QA Microbiology

Paul Norland -- QA Manager

SECTION 3

QUALITY ASSURANCE AUDIT REPORT & STATEMENT

GENEVA LABORATORIES, INC.
GLP AUDIT SCHEDULE REPORT, TEST ID AND CERTIFICATION

SPONSOR: Global Health Solutions
P.O. Box 133/1360 Redmond Circle, NW
Rome, GA 30162

TEST ARTICLE: CurX Antimicrobial Wound Dressing
Lot No.: 15F10E3

NATURE OF STUDY: Cytotoxicity Evaluation
Agar Overlay/L929 Mouse Fibroblast
[ANSI/AAMI/ISO 10993-5:2009(R)2014]

REFERENCE: Geneva Laboratories Procedure No.: CC1002Q; Add. ISO-01C

TEST SYSTEM: L929 Mouse Fibroblast Cells (serial subculture)

TEST STATUS: Study Initiated: 02-22-2016
Test Initiated: 03-02-2016
Test Completed: 03-04-2016
Study Completed: 03-14-2016

AUDIT DATES: See Table I

COMMENTS INCLUDING DEVIATIONS AND PROBLEMS: Under the conditions of the study, the test article meets test acceptance requirements

My review of the study documents indicates that the facilities, equipment, personnel, methods, practices, records and controls are in conformance with the GLP Regulations. This final report accurately describes the methods and standard operating procedures used and the raw data generated during the course of the study.

The copies of the protocols and records of Quality Assurance inspections have been transferred to the Geneva Laboratories GLP archive and will be maintained as long as indicated in 21 CFR Part 58 §58.195 paragraph a) and b).

QA AUDITOR: E Jane Lewis DATE: 03-14-2016

QA MANAGEMENT: Paul Mitchell DATE: 03/14/2016

TABLE I

QUALITY ASSURANCE AUDIT DATES

INSPECTED BY INSPECTION DATE	STUDY SEGMENT INSPECTED	DATE FINDINGS WERE WRITTEN FOR MANAGEMENT AND STUDY DIRECTOR
E.J.L./03-02-2016	Sample Preparation	03-02-2016
E.J.L./03-02-2016	Test Exposure	03-02-2016
E.J.L./03-03-2016	Cellular Fixation	03-03-2016
E.J.L./03-04-2016	Grading	03-04-2016
E.J.L./03-11-2016	Raw Data Review	03-11-2016
E.J.L./03-14-2016	Final Report Review	03-14-2016

*E.J.L. - E. Jane Lewis

QA AUDITOR: E Jane Lewis DATE: 03-14-2016

QA MANAGEMENT: [signature] DATE: 03/14/2016

SECTION 4

COMPLIANCE/ARCHIVE STATEMENTS

GLP0001*

GENEVA LABORATORIES, INC.
STUDY DIRECTOR COMPLIANCE STATEMENT

SPONSOR: Global Health Solutions
P.O. Box 133/1360 Redmond Circle, NW
Rome, GA 30162

PROTOCOL: Cytotoxicity Evaluation
Agar Overlay/L929 Mouse Fibroblast
[ANSI/AAMI/ISO 10993-5:2009(R)2014]

TEST ARTICLE: CurX Antimicrobial Wound Dressing
Lot No.: 15F10E3

STUDY INITIATION DATE: 02-22-2016 STUDY COMPLETION DATE: 03-14-2016

After a review of the pertinent raw data, I am led to conclude the test results were accurately recorded and verified, correctly analyzed, interpreted and all applicable GLP Regulations of 21 CFR Part 58 for Non-Clinical Laboratory Studies were followed.

All raw data, documentation, protocols, specimens and final reports are retained for orderly storage and expedient retrieval as recommended in the 21 CFR Part 58 §58.190.

STUDY DIRECTOR: Sharon Smith DATE: 03-14-2016
Microbiology

GLP0002*

GENEVA LABORATORIES, INC.
GLP COORDINATOR ARCHIVE STATEMENT

SPONSOR: Global Health Solutions
P.O. Box 133/1360 Redmond Circle, NW
Rome, GA 30162

PROTOCOL: Cytotoxicity Evaluation
Agar Overlay/L929 Mouse Fibroblast
[ANSI/AAMI/ISO 10993-5:2009(R)2014]

TEST ARTICLE: CurX Antimicrobial Wound Dressing
Lot No.: 15F10E3

STUDY INITIATION DATE: 02-22-2016 STUDY COMPLETION DATE: 03-14-2016

For the purpose of information retrieval, we are informing you of our storage procedure of specimens and records.

Specimens and a copy of the final report are stored in the archives of Geneva Laboratories, Inc. Fragile specimens will be retained so long as the quality of the preparation affords evaluation.

Raw data for the above listed test compiled by Geneva Laboratories is stored at Geneva Laboratories (or an alternate archive location) for not less than five (5) years.

GLP COORDINATOR: Lani Smith DATE: 03.14.2016