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

GLOBAL HEALTH SOLUTIONS

LM-200-159A
Lot No.: Pilot/Test Batch-11/16/17

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

March 15, 2018

JN18B1477

JN18B1477

TABLE OF CONTENTS

			NO. OF PAGES
SECTION 1	--	Cytotoxicity Test Protocol	6
SECTION 2	--	Test Report	5
		Study Personnel	1
SECTION 3	--	Quality Assurance Audit Report	2
SECTION 4	--	Compliance/Archive Statements	2

TOTAL PAGES IN REPORT: 16

SECTION 1

TEST PROTOCOL

GLP0002*

JN18B1477

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

GLP PROTOCOL

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

SPONSOR: Global Health Solutions
5959 Topanga Canyon Blvd. #170
Woodland Hills, CA 91367

P.O. No.: PO000000026

TEST ARTICLE: LM-200-159A

LOT/ID: Pilot/Test Batch-11/16/17

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

STUDY DIRECTOR: _____ STUDY INITIATION DATE: 2-23-2018
Vince Lazzaroni
Study Director/Microbiology
Geneva Laboratories, Inc.

SPONSOR: _____ DATE: 2/23/2018
Global Health Solutions

18-018

GENEVA LABORATORIES, INC.

PROTOCOL FOR CYTOTOXICITY EVALUATION
(Fluid Extract/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 (Fluid Extract/L929 Mouse Fibroblast)
Geneva Laboratories Procedure No.: CC1001*
Add. ISO-01*

2). PURPOSE

To assess biological reactivity of mammalian cells (grown in culture) after exposure to extracts prepared from either component materials or finished medical products.

3). IDENTIFICATION OF

	Name	CAS/Code (Lot No.)
Test Article:	LM-200-159A	Pilot/Test Batch-11/16/17
Positive Control:	Amber Latex Tubing	P/N 62996-473; VWR Scientific
Negative Control:	HDPE Sheet Stock	P/N 46009; U.S. Plastic Corp.
Extractant Negative Control:	MEM w/5% FBS (or Alternate Extraction Vehicle)	Current Stock (varies by test date)

4). SPONSOR

Global Health Solutions
5959 Topanga Canyon Blvd. #170
Woodland Hills, CA 91367
ATTN: Mr. Bradley Burnam

*Denotes current revision

5). TEST FACILITY

Geneva Laboratories, Inc.
1001 Proctor Drive
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 plates, are labeled with the appropriate cell line, cell passage number and date of passage.

8). DESCRIPTION OF EXPERIMENTAL DESIGN & BIAS CONTROL

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

A. Preparation of Extracts

1. Routine extraction of the test article and controls will be conducted aseptically and will generally employ serum supplemented media, i.e. Minimum Essential Medium (MEM) with 5% Fetal Bovine Serum (FBS). An antibiotic/antimycotic is incorporated in the media to limit outgrowth of adventitious microorganisms.

2. The ratio of sample (test article/control) to extracting medium will generally follow the guidance described in USP (current revision), Section <88>,"Biological Reactivity Tests, In-Vivo," Table 3 (which significantly concurs with ISO 10993-12 "Biological evaluation of medical devices - Part 12: Sample preparation and reference materials").

3. The general extraction conditions applied will be twenty-four (24) to twenty-six (26) hours at 37°C.

   NOTE: Where the test article has been identified as a permanent implant, the extraction interval will be extended to seventy-two (72) ± two (2) hours.

4. The use of an alternate extraction approach is acceptable provided it meets ISO recommendations [ANSI/AAMI/ISO 10993-5:2009/(R)2014) & ISO 10993-12].

5. The rationale for the use of a particular extraction approach is to be documented in an appropriate format (e.g. GLP Requisition Form, Uniform Biocompatibility Extraction Guidance Form or Protocol Amendment).

B. Test Exposure

1. Immediately after completion of the extraction interval, the extract solution will be removed from contact with the sample.

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

3. The entire volume of media in each of the test wells will be carefully drawn off (so as not to disturb the cells) and replaced with an equivalent volume of the extract/extractant (in triplicate).

   a. If the extraction solution does not contain media, it must be diluted in culture media at the time of exposure.

   b. Serial dilutions of the positive control extract will also be tested to determine the negative reactivity endpoint, as applicable.

      NOTE: Serial dilutions of the test article extract may also be evaluated at sponsor request.

C. Bias Control

Bias is controlled either by testing the entire test article (whole assembly), or if not feasible, by sampling the various materials proportionally to their representation in the whole assembly. 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. After supplanting the growth medium with the extract preparation, no further supplementation will occur.

3. In both situations, the volume used per test well will be in accordance with the guidance described in "Basic Laboratory Techniques in Cell Culture", CDC, 1981.

B. Environmental Controls

1. The test system (monolayer plates) will be held at 37 ±1°C for the three (3) day 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 intervals of twenty-four (24) and seventy-two (72) ±one (1) hours after exposure, the test plates will be microscopically examined for indication of cellular response.

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. Trypan Blue) will be employed at the final reading to help visualize the response.

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). 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.

13). 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

P.O. NO.: PO000000026

REPORT TO: Mr. Bradley Burnam
Global Health Solutions
5959 Topanga Canyon Blvd. #170
Woodland Hills, CA 91367

TEST ARTICLE: LM-200-159A

LOT NO.: Pilot/Test Batch-11/16/17

DATE RECEIVED: 02-22-2018

TEST INITIATION DATE: 03-05-2018 TEST COMPLETION DATE: 03-09-2018

TEST PROCEDURE: Cytotoxicity Test-Fluid Extraction/L929 Mouse Fibroblast
Ref. Geneva Laboratories Proc. No.: CC1001; Rev. N
ISO Method Add. No. ISO-01; Rev. H
ANSI/AAMI/ISO 10993-5:2009/(R)2014 Biological evaluation of medical devices - Part 5: Test for in vitro cytotoxicity (GLP)

OBJECTIVE: To assess the biological reactivity of a mammalian cell culture to extract solutions prepared from component materials or finished medical products.

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

X Meets test acceptance requirements
N/A Meets test acceptance requirements in part only
N/A Does not meet test acceptance requirements

For a detailed description of test methods and findings, see pages 2-5.

ANALYST: _____ DATE: 03-13-2018

ACCEPTED BY: _____ DATE: 3.14.2018
Technical Reviewer

Q.A. SIGNATURE: _____ DATE: 03-14-2018

CONTROL ARTICLES:

Positive Control -- Amber Latex Tubing
Lot W034-070 (VWR)

Negative Controls -- HDPE Sheet Stock
Lot 10152013 (U.S. Plastics)

STABILITY: The material positive and negative controls (latex tubing and HDPE sheet) are solid items with no known stability concerns when held at room temperature conditions. The extract negative control ("MEM") has a shelf life of two (2) weeks from the batch date when held at refrigerated conditions. The extract preparations from these items as well as the test article are tested immediately upon completion of the extraction phase unless their stability in storage is otherwise demonstrated.

TEST SYSTEM: The test was performed using mouse fibroblast cells obtained from Diagnostic Hybrids (Cell Line L929, Lot 011509). Cells had been subcultured using a growth medium, MEM w/10% FBS. The cells were held at 37°C ±1°C/5% CO_2 during the growth phase.

The passage number/date of the subculture utilized for this test was: p707/030518-2

TEST METHODS:

A. Extract Preparation

The test article was prepared and extract volume determined as recommended in: Current USP, Section <88>, Table 3
Test Article per Extract Ratio: 38.1 cm^2/12.7 mL

The test article was placed in a sterile extraction container*. Then, under laminar flow conditions, the predetermined volume of the extracting medium was added.

Extractant/Diluent Used: MEM w/5% FBS Lot No. 03052018

*NOTE: Testing of some articles may be conducted on the fluid pathway only, other articles may be in liquid form. See Section E for special test conditions.

TEST METHODS (cont.):

Positive and negative control materials were prepared and extract volumes determined as recommended in current USP, Section <88>, Table 3.

Material Positive Control per Extract Ratio: 61.4 cm^2/20.5 mL
Material Negative Control per Extract Ratio: 60.0 cm^2/20.0 mL
Extractant Negative Control Test Volume: 20.0 mL

After preparation, the test article and controls were immediately placed in a 37 ±1°C incubator to begin extraction.

Extraction Date: 03-05-2018
Extraction Interval: 24 Hours

After extraction and immediately prior to sampling for cellular exposure, the test article was agitated in the extractant to assure a homogenous test suspension. The condition of the extract/test article was noted at this time as unchanged.

B. Cell Preparation

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

Then, just prior to exposure, each of the plates was microscopically examined for possible contamination and the observed level of confluency.

Percentage of confluency at time of use: 80-85%

Once found to meet the acceptance criteria for use in the test, individual wells were numbered (in triplicate) to represent the controls, including a positive control dilution series, and the test article undiluted.

C. Cell Exposure

The subculture medium was carefully removed from the wells and replaced with an equivalent amount of extract/extractant. Exposure time and the date were identified on the plates used. Each of the test plates was then placed in the 37° ±1°C/5% CO_2 incubator to initiate the exposure interval.

Exposure Date: 03-06-2018

D. Observations

Microscopic readings for cellular response were performed at 24 hours (±1 hour) and 72 hours (±1 hour) after exposure.

At least one (1) well was stained with Trypan Blue at the final reading to help determine "percent lysed".

See Table I below for grading guidelines.

TABLE I

Grade	Reactivity	Conditions of Cell Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis.
1	Slight	No more than 20% of the cells are round, loosely attached and without intracytoplasmic granules; occasional lysed cells may be present.
2	Mild	Not more than 50% of the cells are round and devoid of intra-cytoplasmic granules; cell lysis and empty areas between cells may be present.
3	Moderate	Not more than 70% of the cell layers contain rounded cells and/or are lysed.
4	Severe	Nearly complete destruction of the cells' layers.

E. Additional Methods

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

Sample was applied to one side only of each of two glass slides and spread evenly across the surface with sterile gauze. Total mass applied was 0.1444 g.

RESULTS: See Table II for the cellular response to extracts from the controls and test sample.

TABLE II

	24 Hours			72 Hours			Trypan Blue % Lysed
Material Positive Control	4	4	4	4	4	4	100%
Material Negative Control	0	0	0	0	0	0	0%
Extract Negative Control	0	0	0	0	0	0	0%
Test Article	2	2	2	1	1	1	5%

DATE READ: 03-07-2018 03-09-2018
READ BY: Daryl Meyer Daryl Meyer

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 article are interpreted according to 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 were made.

CONCLUSION:

The Grade 1 response to 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 -- Microbiology Study Director

Daryl Meyer -- Microbiology Supervisor/Analyst

E. Jane Lewis -- QA Scientist

Paul Norland -- QA Manager

SECTION 3

QUALITY ASSURANCE AUDIT REPORT & STATEMENT

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

SPONSOR: Global Health Solutions
5959 Topanga Canyon Blvd. #170
Woodland Hills, CA 91367

TEST ARTICLE: LM-200-159A
Lot No.: Pilot/Test Batch-11/16/17

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

REFERENCE: Geneva Laboratories Procedure No.: CC1001N, Add. ISO-01H

TEST SYSTEM: L929 Mouse Fibroblast Cells (serial subculture)

TEST STATUS: Study Initiated: 02-23-2018
Test Initiated: 03-05-2018
Test Completed: 03-09-2018
Study Completed: 03-15-2018

AUDIT DATES: See Table I

COMMENTS INCLUDING DEVIATIONS AND PROBLEMS: The test article extract is considered non-cytotoxic and meets ISO acceptance criteria of no more than grade 2 reactivity.

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). The Quality Assurance Department is independent of and impartial to: The Testing Department, inspection of data and reporting of the results pertaining to this study.

QA AUDITOR: E Jane Lewis DATE: 03-15-2018

QA MANAGEMENT: [signature] DATE: 03/15/2018

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./03-02-2018	Sample Preparation	03-02-2018
E.J.L./03-05-2018	Extraction	03-05-2018
E.J.L./03-06-2018	Cell Exposure	03-06-2018
E.J.L./P.N. 03-09-2018	Final Reading	03-09-2018
E.J.L./03-14-2018	Raw Data Review	03-14-2018
E.J.L./03-15-2018	Final Report Review	03-15-2018

*E.J.L. - E. Jane Lewis
P.N. - Paul Norland

QA AUDITOR: E Jane Lewis DATE: 03-15-2018

QA MANAGEMENT: [signature] DATE: 03/15/2018

SECTION 4

COMPLIANCE/ARCHIVE STATEMENTS

GLP0001*

GENEVA LABORATORIES, INC.
STUDY DIRECTOR COMPLIANCE STATEMENT

SPONSOR: Global Health Solutions
5959 Topanga Canyon Blvd. #170
Woodland Hills, CA 91367

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

TEST ARTICLE: LM-200-159A
Lot No.: Pilot/Test Batch-11/16/17

STUDY INITIATION DATE: 02-23-2018 STUDY COMPLETION DATE: 03-15-2018

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: _____ DATE: 3.15.2018
Microbiology

GENEVA LABORATORIES, INC.
GLP COORDINATOR ARCHIVE STATEMENT

SPONSOR: Global Health Solutions
5959 Topanga Canyon Blvd. #170
Woodland Hills, CA 91367

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

TEST ARTICLE: LM-200-159A
Lot No.: Pilot/Test Batch-11/16/17

STUDY INITIATION DATE: 02-23-2018 STUDY COMPLETION DATE: 03-15-2018

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, Inc. (or an alternate archive location) for not less than five (5) years.

GLP COORDINATOR: Rachelle Hoacher DATE: 03-15-2018