

APS Study ID: MFY001
APS Study Type: CY02

Agar Overlay Cytotoxicity Assay (Liquids)

GLP Final Report

Regulatory Compliance:
ISO 10993-05:2009

TABLE OF CONTENTS

1.0	STUDY CONTACT INFORMATION	4
1.1	TITLE:	4
1.2	STUDY NUMBER:	4
1.3	SPONSOR:	4
1.4	SPONSOR REPRESENTATIVE:	4
1.5	TEST FACILITY:	4
1.6	STUDY DIRECTOR:	4
1.7	LEAD QAU:	4
2.0	FINAL REPORT SIGNATURE PAGE	5
3.0	EXECUTIVE SUMMARY	6
4.0	GENERAL STUDY INFORMATION	7
4.1	STUDY INITIATION AND COMPLETION DATES	7
4.2	REGULATORY COMPLIANCE	7
4.3	PURPOSE / OBJECTIVE	7
4.4	REACTIVITY GRADES	8
4.5	ASSAY PERFORMANCE ACCEPTANCE CRITERIA	8
4.6	LIQUID ARTICLE CHARACTERIZATION	8
4.6.1	<i>Test Article(s)</i>	8
4.6.2	<i>Cell Control</i>	8
4.6.3	<i>Negative Control</i>	9
4.6.4	<i>Positive Control</i>	9
4.7	TEST SYSTEM DESCRIPTION AND JUSTIFICATION	9
4.8	METHODS	10
4.8.1	<i>Study Design</i>	10
4.8.2	<i>Cell Preparation</i>	10
4.8.3	<i>Sample Preparation</i>	10
4.8.4	<i>Preparation of the Agar Surface</i>	10
4.8.5	<i>Test Completion</i>	11
5.0	RESULTS	12
6.0	DISCUSSION / CONCLUSION	13
7.0	RECORD STORAGE	13

TABLE OF TABLES

TABLE 1:	INCUBATION	10
TABLE 2:	STAINING	11
TABLE 3:	RESULTS	12

TABLE OF APPENDICIES

APPENDIX A:	PROTOCOL.....	14-31
APPENDIX B:	AMENDMENTS	32
APPENDIX C:	STUDY DEVIATIONS	33
APPENDIX D:	QUALITY ASSURANCE STATEMENT	34-35

ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AOL	Agar Overlay
APS	American Preclinical Services
CdCl ₂	Cadmium Chloride
EMEM5% FBS	Eagle's Minimal Essential Medium with 5% Fetal Bovine Serum
FBS	Fetal Bovine Serum
HDPE	High Density Polyethylene
ISO	International Organization for Standardization
L-929	NCTC clone 929 (derivative of Strain L) from mouse, obtained from ATCC
MFY	Simple Science
PBS	Phosphate Buffered Saline
SPA	Sponsor Protocol Approval
SRF	Study Requisition Form, F-S-GN-SC-010-02
USP	United States Pharmacopeia
2X MEM	Twice concentrated Minimal Essential Medium

1.0 STUDY CONTACT INFORMATION

- 1.1 Title:** Agar Overlay Cytotoxicity Assay (Liquids)
- 1.2 Study Number:** MFY001-CY02
- 1.3 Sponsor:** Simple Science
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2.0 FINAL REPORT SIGNATURE PAGE

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Sarah Howard, BA

Test Facility Management: *Jessica Zak* Date: 032814
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3.0 EXECUTIVE SUMMARY

Purpose/Objectives: The purpose the Agar Overlay Cytotoxicity assay was to provide general information on the ability of a Sponsor provided test article to cause a cytotoxic response of L-929 cells through an agar barrier.

Test Device: According to the Sponsor, the test article consisted of CleanSmart 01. The liquid test article was used to saturate 3 filter discs. No preparation was required and the liquid sample was tested neat.

Methods: The liquid controls and test article were used to saturate three filter discs. The maintenance culture media was removed from the cells plated into 60 mm dishes and replaced with 3.5 mL of 2X MEM + agar mixture. The test article and controls were placed directly onto the agar surface in triplicate. The cell control consisted of a single dish with the 2X MEM + agar mixture. The prepared dishes were incubated at 37 ± 1 °C with $5 \pm 1\%$ CO₂ and $85 \pm 15\%$ humidity for 24-25 hours. After incubation, the outline of each filter disc was traced. The articles were removed and the dishes were flooded with 3.0 mL of 0.01% neutral red stain. The stained dishes were incubated at 37 ± 1 °C with $5 \pm 1\%$ CO₂ and $85 \pm 15\%$ humidity for 60 ± 10 minutes. The stain was discarded and the cells were evaluated under a light microscope.

Results: Under the conditions of this study, the test article (CleanSmart 01) received a cytotoxicity grade of '0' and was considered Non-Cytotoxic.

Discussion/Conclusion: Based on the scope of this study and the grading system described in section 4.4, the microscopic evaluation of the cells revealed that cell control received a grade of '0' and was considered Non-Cytotoxic. The negative control article received grade of '0' and was considered Non-Cytotoxic. The positive control received a grade of '3' and was considered Cytotoxic. The results met the assay performance acceptance criteria and the test was considered valid.

4.0 GENERAL STUDY INFORMATION

4.1 Study Initiation and Completion Dates

Protocol Approved by the Study Director	<i>In Vitro</i> Completion	Final Report
03/12/2014	03/19/2014	03/27/2014

4.2 Regulatory Compliance

The study was conducted in compliance to the International Organization for Standardization (ISO) 10993: Biological Evaluation of Medical Devices, Part 5- Tests for *In Vitro* Cytotoxicity (2009).

It is the intention of Simple Science, through the conduct of this study, to generate data that may be submitted to regulatory authorities. The Test Facility followed all requirements specified in the approved Protocol, Protocol Amendments, Test Facility Standard Operating Procedures (SOPs) and 21 CFR Part 58 (FDA). Deviations to the approved protocol are listed in Appendix C. There were no deviations that affected the quality or integrity of the study or the interpretation of the results in the report.

The APS Quality Assurance Unit, in accordance with the Test Facility's SOPs, inspected the study conduct and Final Report.

4.3 Purpose / Objective

The purpose the Agar Overlay Cytotoxicity assay was to provide general information on the ability of a Sponsor provided test article to cause a cytotoxic response of L-929 cells through an agar barrier. [REDACTED]

4.4 Reactivity Grades

Grade	Reactivity	Interpretation	Description of Reactivity Zone
0	None	Non-Cytotoxic	No detectable zone around or under specimen
1	Slight	Non-Cytotoxic	Some malformed or degenerated cells under specimen
2	Mild	Non-Cytotoxic	Zone limited to area under specimen
3	Moderate	Cytotoxic	Zone extending specimen size up to 1.0 cm
4	Severe	Cytotoxic	Zone extending farther than 1.0 cm beyond specimen

4.5 Assay Performance Acceptance Criteria

- 1.) Triplicate control/test article wells produced similar responses (± 1 grade).
- 2.) The cell media control displayed a result of zero.
- 3.) The negative control article produced a non-cytotoxic response in triplicate.
- 4.) The positive control article produced a cytotoxic response in triplicate.

4.6 Liquid Article Characterization

4.6.1 Test Article(s)

Name: CleanSmart 01
Lot Number: Q415013001
Expiration Date: January 2015
Storage: Ambient
Sterilization: Submitted Non-Sterile

4.6.2 Cell Control

Name: Cell Control
Description: Equal amounts of 2X MEM and 2.0% Agar were mixed together. 3.5 mL of the mixtures was added to a single 60 mm dish containing a subconfluent monolayer of L-929 cells.
Stability: Stable under these testing conditions

4.6.3 Negative Control

Name:	Negative Control
Description:	The liquid negative control consisted of EMEM 5% FBS (pH = 7.2). The media was used to saturate 3 filter discs.
Lot Number:	APS030414-2
Expiration Date:	03/18/2014
Storage Conditions:	2- 8 °C (Per Solution Preparation form F-S-IV-GN-OP-003-01)
Stability:	Stable under these testing conditions

4.6.4 Positive Control

Name:	Positive Control
Description:	1.0 mL of a 1 mg/mL cadmium chloride solution was mixed with 9.0 mL of EMEM 5% FBS. The solution was used to saturate 3 filter discs.
Lot Number:	APS020414-1
Expiration Date:	02/04/2015
Storage Conditions:	2 – 8 °C (Per Solution Preparation form F-S-IV-GN-OP-003-01)
Stability:	Stable under these testing conditions

4.7 Test System Description and Justification

NCTC clone 929 (L cell, L-929, derivative of Strain L) L-929 Cells were originally obtained from American Type Culture Collection (ATCC) L-929 is an established cell line that produces reliable and consistent results. Mycoplasma testing was completed from a reputable vendor and the cells were determined to be mycoplasma-free.