TEKNOLOGISK INSTITUT

Re: Guardian Disinfecting Solution

We hereby confirm that Guardian Disinfecting Solution have been tested against vaccinia virus and found effective. The test also covers Corona virus.

2019 nCoV belongs to the group of enveloped viruses and is consistently covered by an activity claim against all enveloped viruses in a quantitative suspension test according to **EN 14476**, where the reference test virus is vaccinia virus.

Sincerely,
Helle Stendahl Andersen
Forretningsleder
Life Science
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www.eurofins.dk

Date
04 June 2012
Our ref.
944006, part 1/SLH

Analytical report

Sample received	Test sample
10 April 2012	Science Guardian

Customer's journal no/ project title: -

Label(s): Batch no. 35021202

Information from client: -

Methods: Specific references are listed with the test results.

Test: Vaccinia virus (HIV SURROGATE)

Results: Results are reported on page 2-14.

Comments: Samples are analysed by the laboratory:
Eurofins Biolab, Italy

Signatures

Sandy Leth
MSc (Pharm)

Final report 2012/667 AMi

SUSPENSION VIRUCIDAL EFFECTIVENESS AGAINST Vaccinia virus (HIV SURROGATE) ON SCIENCE GUARDIAN

Study Program No: 2012/667 AM

Contract No: PPR12012010105

Sponsor: EUROFINS PHARMA A/S
STRANDESPLANADEN 110
DK-2655 VALLENSBAEK STRAND
DENMARK

Test substance: SCIENCE GUARDIAN

eurofins
Eurofins Pharma A/S
Ørnebjergvej 1
2600 Glostrup
Tlf. [illegible]
04. Jan. 2012

Director of the Study: Laura Brambilla
(Laura Brambilla)

Released on: May 28th 2012

Eurofins Biolab S.r.l.
Società con Socio unico sottoposta
a direzione e coordinamento della società
Eurofins Scientific Italia S.r.l.
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C.SOC. € 100.000 i.v.
P. IVA 00762140960
C.F. 03765750157
REA MI 966696
D-U-N-S 429117112
CIT005 4-385

INDEX

COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the studies described in this report have been conducted under my supervision and in compliance with the following standards of Good Laboratory Practice:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organisation for Economic Co- Operation and Development , Paris 1998.

- Legislative decree n. 50 of March the 2nd, 2007. Enforcement of Community Directives 2004/9/CE e 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86 of April the 13th, 2007).

- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent amendments.

- Decree of the Italian Ministry of Health October the 12th 2010, certification N. 121/2010 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

There were no circumstances that may affect the quality or integrity of the study.

STUDY DIRECTOR
(Laura Brambilla)

May 28th 2012
DATE

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved study program and the Standard Operating Procedures of Eurofins Biolab Srl.
The study and/or the test facility were periodically inspected by the Quality Assurance unit according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance unit, personnel independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections have been carried out and reported to the Director of the Study and to Eurofins Biolab's S.r.l. Management:

PHASE OF STUDY	DATE OF INSPECTION / REPORTING
Pre-experimental period	//
Experimental period	//
Post-experimental period	//
Documentation:	
- Study program	April, 30th 2012
- Raw data	May, 28th 2012
- Final report	May, 28th 2012

This report accurately reflects the raw data.

Monica Rossetti
GLP QUALITY ASSURANCE
(Monica Rossetti)

May, 28th 2012
DATE

Eurofins Biolab S.r.l.
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SUMMARY

A series of assays were performed on the test substance SCIENCE GUARDIAN to determine the suspension virucidal effectiveness against *Vaccinia virus (HIV SURROGATE)* for the specific uses provided for the product.
To this purpose the following test was performed:

- **Virucidal activity. Suspension test** in which a viral suspension, *Vaccinia virus Strain WR* ATCC VR-119, were incubated with the test substance in the following conditions test:
  - concentration: neat (90% maximum concentration which can be tested)
  - contact time: 30 minutes
  - interfering substance: none
  - Neutralizer: Medium culture (EMEM) + FBS 5%

After the incubation time, viral suspensions were inoculated in the appropriate cellular monolayer of VERO.
After 3 days, cellular cultures were observed with the inverted microscope for the detection of cytopatic effects (CPE) produced by viral multiplication.

Before checking virucidal activity, following tests to validate the method were performed, in order to verify the compliance to the ASTM E 1052 requirements:
1. CELL CULTURE (medium alone)
2. CYTOTOXICITY CONTROL OF TEST SUBSTANCE (1 part medium + 9 part test substance)
3. VIRUS CONTROL (1 part virus + 9 parts medium)
4. NEUTRALIZATION CONTROL (neutralized test substance + virus)

On the basis of obtained results, in the adopted experimental conditions, the test substance SCIENCE GUARDIAN **causes a reduction of 3.00 Log** against *Vaccinia virus* Strain WR ATCC VR-119 with the test concentration after 30 minutes of contact.

See Experimental Report 2012/667 for more details.

INTRODUCTION

A study was conducted on behalf of EUROFINS PHARMA A/S to demonstrate the suspension virucidal effectiveness against *Vaccinia virus (HIV SURROGATE)*, in accordance with ASTM E 1052 – 11 and Sponsor requirements.
The study was performed at the Test Facility Eurofins Biolab S.r.l. of Vimodrone (MI) – via B. Buozzi n. 2 (Italy).

EXPERIMENTATION	*START*	*END*	*RESEARCHER*
Virucidal activity – suspension test	May 07th 2012	May 10th 2012	C. Meroni

In this report:
- doses are expressed as grams of the test substance per 100 millilitres of water (%)
- the virus titres are expressed as TCID50: 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units.

TERMS AND DEFINITIONS

Virucidal: a chemical agent or a formulation that inactivates viruses under certain conditions.

Virucidal activity: the capability of a product to produce a reduction in the number of viruses under certain conditions.

REFERENCES

ASTM E 1052 – 11: Standard Test Method to assess the activity of microbicides against viruses in suspension.

FILING

The study program, all raw data are filed in the archives of Eurofins Biolab S.r.L for ten years after the issuing of the final report.
No retained sample will be not kept because the Sponsor did not provided stability information and because it consists in an aliquot of the study 2012/665 AM.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

PROCEDURES

All procedures used during this study are recorded in the Eurofins Biolab S.r.L Procedures Manual.

TEST PRODUCT

The test product is an antiseptic (ready-to-use solution).

Product	SCIENCE GUARDIAN
Stability	Not provided
Composition on safety data sheet	Preparation containing an acqueous solution of cationic polymer and surfactant

ANALYSED SAMPLE

The analysed sample, representative of the test substance, consists in an aliquot of the sample of the study 2012/665 AM that was a transparent colourless liquid contained into a white plastic jar.

Batch	3502/202
Manufacturing date	Not provided
Expiry date	Not provided
CoA	Not provided
Receiving	EUITVI-24992
Date	Apr 11th 2012
#ID	12.955-S

The characterisation of the test product is under Sponsor's responsibility

Experimental Report 2012/667 - VIRUCIDAL ACTIVITY AGAINST VACCINIA VIRUS (HIV SURROGATE) – SUSPENSION TEST (ASTM E 1052 – 11)

EXPERIMENTAL PROCEDURE

1. ASSAY SYSTEM

Virus

Vaccinia virus Strain WR ATCC VR-119

The viral suspensions were kept frozen at <–70°C; before their use they were multiplied in the appropriate cellular line, as shown following:

VIRUS STRAINS	HOST CELL LINE	INCUBATION TIME (Days)	INCUBATION TEMPERATURE
Vaccinia virus Strain WR	Vero	3	37±1°C (CO_2 5%)

Cellular debris were removed by means of double centrifugation at low speed, and the surnatant containing the virus was used for the test.

2. CELL LINES, MEDIA AND REAGENTS

Cellular cultures

VERO ATCC CCL-81

The cellular culture was kept frozen at <–70°C; before viral inoculum, it showed itself as confluent monolayer.

Culture Medium and reagent

EMEM:	Eagle's minimal essential medium	BIOWHITTAKER - LONZA
FBS (2%)	Foetal Bovine Serum	BIOWHITTAKER – LONZA
PEN-STREP (1%)	antibiotics	BIOWHITTAKER – LONZA
WFI	Water for injection	EUROSPITAL
PBS	Phosphate Buffer Saline	BIOWHITTAKER - LONZA

3. EQUIPMENT AND MATERIALS

Incubator	ARBORE
Centrifuge	HERAEUS
Deepfreeze	Angelantoni Scientifica
CO_2 incubator	PBI
Vortex	VELP
Chronometer	ARBORE
Micropipettes	GILSON
Microdishes 96 wells	LONZA
Inverted microscope	LEITZ
pHmeter	BECKMAN
Vessel containing water and ice	
Water bath	ARBORE

4. EXPERIMENTAL DESIGN

Test temperature
The test was conducted at 22°C ±2°C.

Test conditions
The test was conducted with the following test conditions:
- final concentration: 90% (maximum concentration which can be tested)
- contact time: 30 minutes

Interfering substance
None.

Neutralizer
Ice-cold Medium culture with 5% FBS.

5. ASSAY EXECUTION

Preparation of the test substance
The test substance was used neat.

Cell control
0.1 ml of Medium culture were transferred in dishes with 96 wells containing the cellular confluent monolayer (>90%) without culture Medium. Each aliquot of Medium was placed onto dishes 4-fold as control of cellular line.
After 1 hour of incubation 0.1ml of culture Medium were added to viral inoculum.
After the incubation period, the cellular culture was observed with inverted microscope to detect any cytopatic effect (CPE) due to viral suspension. After this detection the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method.

Cytotoxicity control
To evaluate any cytopatic effect of the test substance, a cytotoxicity test was conducted by mixing 1 ml of the assay sample with 9 ml of culture Medium. Then serial dilutions 1:1 were performed and 0.1 ml of each dilution of the test substance put on dishes 4-fold.
The outline of the microplate did not receive the viral inoculum and were used as control of cellular line.
After 1 hour of incubation 0.1ml of culture Medium were added to each well.
Immediately after the addition of the test substance and daily for 3 days, the cellular culture were observed with inverted microscope to detect any cytopatic effect (CPE) due to test substance.

Virus control
Serial dilutions 1:10 were prepared with culture Medium, starting from virus stock solution of viral suspension (1 part of virus + 9 part of medium culture). 0.1 ml were transferred in dishes with 96 wells containing the cellular confluent monolayer (>90%) without culture Medium. Each dilution of viral suspension was placed onto dishes 4-fold.
The outline of the microplate did not receive the viral inoculum and were used as control of cellular line.
After 1 hour of incubation 0.1ml of culture Medium were added to viral inoculum.
In parallel the same test described above, was performed with the neutralize alone instead of the test substance neutralized and also with a control fluid PBS (validation of the neutralization).
After the incubation period, the cellular culture was observed with inverted microscope to detect any cytopatic effect (CPE) due to viral suspension. After this detection the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method.

Neutralization control

To evaluate the effective neutralization dilution, another cytotoxicity test was conducted by mixing 1 ml of the assay sample with 9 ml of neutralizer (EMEM + 5%FBS). Serial dilutions 1:10 of this test mixture with culture medium were performed; then 0.1 ml of the diluted stock viral suspension (containing 1000-500 infectious units) were added into the neutralized sample and 0.1 ml of each dilution put on dishes with the cellular confluent monolayer (>90%), 4-fold.
The outline of the microplate did not receive the viral inoculum and were used as control of cellular line.
After the appropriate incubation period, the cellular culture was observed with inverted microscope to detect any cytopatic effect (CPE) due to viral suspension. After this detection the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method.

Virucidal test

1 ml of viral suspension was mixed with 9 ml of the assay sample and left in contact for the test contact time.
At the end of contact time, 1 ml of the solution was transferred into 4.5 ml of neutralizer (EMEM + 5%FBS) in an iced bath; then serial dilutions 1:10 with culture Medium were performed.
0.1 ml of this solution was put onto dishes in 4-fold in the microplate containing the cellular confluent culture (>90%).
The outline of the microplate did not receive the viral inoculum and were used as control of cellular line.
After 1 hour of incubation at 37°C ±1°C 0.1 ml of culture Medium were added to the viral inoculum.
After the appropriate incubation period, the cellular culture was observed with inverted microscope to detect any cytopatic effect (CPE) due to viral suspension. After this detection the infecting activity ($TCID_{50}$ evaluation) was calculated by means of Spearman – Karber method.

6. CALCULATIONS AND EXPRESSION OF THE RESULTS

Determination of $TCID_{50}$

The infecting activity was determined by means of Spaerman – Karber method that uses the following formula to calculate the value of $TCID_{50}$:

$$-\text{Log } TCID_{50} = -(-x_0) - \{[R/100] - 0.5\} \times \text{Log dilution factor}$$

where:

x_0 = Log of the lowest dilution with 100% of positive reaction (CPE)
R = sum (%) of positive cultures

Log $TCID_{50}$ values are rounded to two significant ciphers. Rounding is automatically performed by excel datasheet.

Evaluation of the virucidal activity

The virucidal activity of the product test solution was performed for each exposure time. The Log reduction was performed by subtracting the logarithmic titre $TCID_{50}$ at any test point from the logarithmic titre $TCID_{50}$ of the virus control.

RESULTS

Cytotoxicity control
The test substance is slightly cytotoxic with the test concentrations adopted. However the level of cytotoxicity didn't affect the evaluation of product efficacy.

Virucidal activity
The results obtained in the adopted experimental conditions are reported at the Attachment #1.
The results of Log reduction at the different contact times are reported following and at the Attachment #1:

Virus test	**Log reduction**
	90%
	30 minutes
***Vaccinia virus* Strain WR ATCC VR-119**	3.00

DEVIATIONS

The study did not undergo deviations compared to the study program.

CONCLUSIONS

On the basis of obtained results, in the adopted experimental conditions, the test substance SCIENCE GUARDIAN **causes a reduction of 3.00 Log** against *Vaccinia virus* Strain WR ATCC VR-119 with the test concentration after 30 minutes of contact.

ATTACHMENTS

ATTACHMENT	TITLE	NUMBER OF PAGES
N.1	RAW DATA: EXPERIMENTATION 2012/667	2

eurofins | biolab	Standard Test Method to assess the activity of microbicides against viruses in suspension
Mod. PS/MIC/074.C	Norma (Standards): ASTM E1052 – 11
Rev.0	Pagina *(Page)* 1 / 2

Data inizio *(Started on)*: 07/05/12

ID. studio (ID. Study): 2012/667 AM

ID Campione *(ID sample)* : 12.955-S

Controllo coltura cellulare *(Cell culture control)*

Condizioni testate *(Test condition)*		Diluizione(-Log) *(Dilution(-Log))*							
		1	2	3	4	5	6	7	8
medium alone	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	Endpoint	0	0	0	0	0	0	0	0

-Log TCID50 -0.50

Citotossicità *(Cytotoxicity)*

Condizioni testate *(Test condition)*		Diluizione(-Log) *(Dilution(-Log))*							
		1	2	3	4	5	6	7	8
SCIENCE GUARDIAN 90.0%	1	4	4	0	0	0	0	0	0
	2	4	3	0	0	0	0	0	0
	3	4	3	1	0	0	0	0	0
	4	4	4	0	0	0	0	0	0
	Endpoint	100	100	25	0	0	0	0	0

-Log TCID50 -2.75

Virus Control *(Virus control)*
Vaccinia virus Strain WR ATCC VR-119

Condizioni testate *(Test condition)*		Diluizione virale(-Log) *(Viral dilution(-Log))*							
		1	2	3	4	5	6	7	8
VIRUS CONTROL Vaccinia virus Strain WR ATCC VR-119	1	4	4	4	4	3	2	1	0
	2	4	4	4	4	4	2	0	0
	3	4	4	4	4	4	2	0	0
	4	4	4	4	4	3	0	0	0
	Endpoint	100	100	100	100	100	75	25	0

-Log TCID50 -6.50

Sigla tecnico *(Technician signature)*:

Sigla Approvazione *(Approval signature)*:

Data fine *(Finished on)*: 10/05/12

Data *(Date)* : 10/05/12

eurofins biolab	Standard Test Method to assess the activity of microbicides against viruses in suspension
Mod. PS/MIC/074.C	Norma (Standards): ASTM E1052 – 11
Rev.0	Pagina *(Page)* 2 / 2

Data inizio *(Started on)*: 07/05/12

ID. studio (ID. Study): 2012/667 AM

ID Campione *(ID sample)* : 12.955-S

Controllo neutralizzazione *(Neutralization control)*
Vaccinia virus Strain WR ATCC VR-119

Condizioni testate *(Test condition)*		Diluizione virale(-Log) *(Viral dilution(-Log))*							
		1	2	3	4	5	6	7	8
Control	1	4	4	2	0	0	0	0	0
PBS	2	4	4	2	0	0	0	0	0
	3	4	4	3	1	0	0	0	0
	4	4	4	2	0	0	0	0	0
	Endpoint	100	100	100	25	0	0	0	0

-Log TCID50 -3.75

Condizioni testate *(Test condition)*		Diluizione virale(-Log) *(Viral dilution(-Log))*							
		1	2	3	4	5	6	7	8
Neutralizer alone	1	4	4	1	0	0	0	0	0
EMEM + FBS 5%	2	4	3	2	0	0	0	0	0
	3	4	4	2	0	0	0	0	0
	4	4	4	2	0	0	0	0	0
	Endpoint	100	100	100	0	0	0	0	0

-Log TCID50 -3.50

Condizioni testate *(Test condition)*		Diluizione virale(-Log) *(Viral dilution(-Log))*							
		1	2	3	4	5	6	7	8
SCIENCE GUARDIAN	1	4	2	1	0	0	0	0	0
90%	2	4	4	2	0	0	0	0	0
EMEM + FBS 5%	3	4	3	0	0	0	0	0	0
	4	4	4	2	1	0	0	0	0
	Endpoint	100	100	75	25	0	0	0	0

-Log TCID50 -3.50

Test virucida *(Virucidal test)*
Vaccinia virus Strain WR ATCC VR-119

Condizioni testate *(Test condition)*		Diluizione virale(-Log) *(Viral dilution(-Log))*							
		2	3	4	5	6	7	8	9
SCIENCE GUARDIAN	1	4	2	0	0	0	0	0	0
90.0%	2	4	0	0	0	0	0	0	0
30 min	3	4	3	1	0	0	0	0	0
None (interfering substance)	4	4	2	0	0	0	0	0	0
	Endpoint	100	75	25	0	0	0	0	0

-Log TCID50 -3.50 Reduction 3.00

Sigla tecnico *(Technician signature)*:

Sigla Approvazione *(Approval signature)*:

Data fine *(Finished on)*: 10/05/12

Data *(Date)* : 10/05/12