Merida®

Wrocław 28.09.2009

OŚWIADCZENIE

Merida Sp. z o.o. z siedzibą we Wrocławiu przy ul. Karkonoskiej 59 oświadcza, że produkt MERIDA DESMED (nasączone ściereczki do czyszczenia i dezynfekcji rąk i powierzchni) działa bójczo na wirus H1N1, zgodnie z wynikami stosownych badań oraz oświadczeniem producenta.

MIEJSCE I DATA WYDANIA:	Wrocław, 28.09.2009
NAZWISKO, STANOWISKO , PODPIS:	Zdzisław Gołębiowski Wiceprezes Zarządu

MERIDA Sp. z o.o.
wiceprezes
Zdzisław Gołębiowski

Merida
Sp. z o.o.
ul. Karkonoska 59
53-015 Wrocław
tel. 339-78-88

Pluswipes Ltd
The Wet Wipes Specialists
Pywell Road
Willowbrook East Industrial Estate
Corby
Northants NN17 5XJ
Tel +44 (0) 1536 408085
Fax +44 (0) 1536 408789
email sales@pluswipes.co.uk
web www.pluswipes.co.uk

28 September 2009

Pluswipes confirms that the H1N1 test results apply to the product Merida Desmed issued to MERIDA Sp. z o.o. KARKONOSKA 59, 53-015 WROCLAW, POLAND.

Signed.....

Andy Lockley
Sales Director

MICROBIOTEST,INC
The Microbiology and Virology Laboratory

FINAL REPORT

EN 14476: Virucidal Quantitative Suspension Test for Chemical Disinfectants and Antiseptics Used in Human Medicine

Human Influenza A Virus (H1N1)

TEST AGENTS:
PWRB001 (2)
PWRB001 (6)

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
693-102

Submitted to:
Pluswipes Ltd
Pywell Road
Willowbrook East Industrial Estate
Corby, Northants, NN17 5XJ
United Kingdom

105B Carpenter Drive, Sterling, VA 20164 • 703-925-0100 • Fax: 703-925-9366
www.microbiotest.com

TABLE OF CONTENTS

COMPLIANCE STATEMENT

This study meets the requirements for 21 CFR § 58 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Zheng Chen, Tien V. Mai, Jennifer Bichan, Salimatu L. Jibril

Study Director: MICROBIOTEST

S. Steve Zhou, Ph.D. Date 08/27/2009

QUALITY ASSURANCE UNIT STATEMENT

Title: EN 14476: Virucidal Quantitative Suspension Test – Human Influenza A Virus (H1N1)

The Quality Assurance Unit of MICROBIOTEST has inspected the Project Number 693-102 in compliance with current Good Laboratory Practice regulations (21 CFR § 58).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

PHASE INSPECTED	DATE OF INSPECTION	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO MANAGEMENT
Protocol	08/25/09	08/26/09	08/26/09
In-Process	08/18/09	08/26/09	08/26/09
Final Report	08/25/09	08/26/09	08/26/09

Felicia L. Sellers
Manager, Quality Assurance Unit

8/27/09
Date

TEST SUMMARY

TITLE: EN 14476: Virucidal Quantitative Suspension Test – Human Influenza A Virus (H1N1)

STUDY DESIGN: This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (See Appendix).

TEST MATERIALS:

1. PWRB001 (2); Lot No. FF 590/2, received at MICROBIOTEST on 07/06/09, and assigned DS No. 10169

2. PWRB001 (6); Lot No. FF 659/1, received at MICROBIOTEST on 07/06/09, and assigned DS No. 10170

SPONSOR:

Pluswipes Ltd
Pywell Road
Willowbrook East Industrial Estate
Corby, Northants, NN17 5XJ
United Kingdom

TEST CONDITIONS

Challenge virus:

Human Influenza A Virus (H1N1), A/PR/8/34, Charles River Laboratories

Host:

MDCK cells, ATCC CCL-34

Neutralizer:

MEM + 1% Fetal bovine serum + 0.5% Lecithin

Contact Time(s):

5 minutes

Contact Temperature:

20 ± 1C (actual 21C)

Dilution:

Ready to use

Test agent application:

Suspension test – 1 mL of stock virus was combined with 1 mL of 10X Interfering substance and added to 8 mL of test agent

Interfering condition:

3.0 g/L BSA + 3.0 mL/L erythrocytes (dirty)

Media and reagents:

MEM + 1 µg/mL Trypsin
Phosphate buffered saline
MEM + 1% Fetal bovine serum + 0.5% Lecithin
Sheep blood
Sephacryl column

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164. Testing was initiated on 08/14/09 and was completed on 08/19/09. The study director signed the protocol on 08/11/09. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where:

m = the logarithm of the titer relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

The viral titer of each sample is reported ± the 95% confidence intervals. The standard error, σ_m, was calculated using the following formula:

$$\sigma_m{}^2 = d_f{}^2 \sum \frac{p_i(1-p_i)}{(n_i - 1)}$$

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

where:

d_f = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
σ_m = the standard error
n_i = number of replicates at dilution i

and $\sum$ denotes the summation over dilutions beginning at the k^{th} dilution. The 95% confidence limit is $m \pm 1.96\sigma_m$.

When a sample contains a low concentration of virus there is a discrete probability that if only a fraction of the sample is tested for virus, that fraction will test negative due to random distribution of virus throughout the total sample. The probability, p, that the sample analyzed does not contain infectious virus is expressed by: $p = [(V-v)/V]^y$, where V is the total volume of the container, v is the volume of the fraction being tested, and y is the absolute number of infectious viruses randomly distributed in the sample. If V is sufficiently large relative to v, the Poisson distribution can approximate p:

$$P = e^{-cv} \qquad \text{or} \qquad c = -[\text{Ln}(P)] / v$$

Where c is the concentration of infectious virus and v is the total sample volume. The amount of virus which would have to be present in the total sample in order to achieve a positive result with 95% confidence (p = 0.05) is calculated as

$$c = -[\text{Ln}(0.05)] / v = 3 / v$$

If all n wells are negative, the virus titer after the process is considered to be less than or equal to this value. The total volume of sample assayed is v = v'nd, where v' is the test volume in a well, n is the number of wells per sample, and d is the sample dilution.

RESULTS

Results are presented in Tables 1-4.

For all tables:

C/y = Cytotoxicity observed in y wells inoculated; viral cytopathic effects (CPE) could not be determined
0/y = 0 wells out of y wells inoculated exhibited positive virus, no CPE detected, no cytotoxicity or bacterial contamination was observed in any of the wells inoculated
x/y = x wells out of y wells inoculated exhibited positive virus, CPE detected
n.d.= not determined
NA = Not applicable

The Viral load was determined in the following manner:

Viral Load (log_{10} $TCID_{50}$) = Titer (log_{10} $TCID_{50}$/mL) + Log_{10}[Volume (mL)]

The log_{10} Reduction Factor was calculated in the following manner:

Log_{10} Reduction Factor = Initial viral load (Log_{10}) – Output viral load (Log_{10})

The 95% confidence interval (CI) of the LRF was calculated in the following manner:

$(CI_{LRF})^2 = (CI_{initial\ load})^2 + (CI_{output\ load})^2$

RESULTS (continued)

Table 1 - Test Results

(Interfering substance: 3.0 g/L BSA + 3.0 mL/L erythrocytes)

Dilution of the neutralized sample (log_{10})	PWRB001 (2) (T = 0 min)*	PWRB001 (2) (T = 5 min)		PWRB001 (6) (T = 0 min)*	PWRB001 (6) (T = 5 min)	
		titration	*large volume*		*titration*	*large volume*
-1	4/4[a]	C/4	n.d.	4/4[a]	C/4	n.d.
-2	4/4[a]	C/4		4/4[a]	C/4	
-3	4/4	0/4	0/48	4/4	0/4	0/48
-4	4/4	0/4	n.d.	4/4	0/4	n.d.
-5	4/4	0/4		4/4	0/4	
-6	4/4	0/4		4/4	0/4	
$TCID_{50}$/mL (log_{10})	≥ 6.50 ± 0.00	≤ 2.83	≤ 1.80	≥ 6.50 ± 0.00	≤ 2.83	≤ 1.80
Volume of sample (mL)	10	10		10	10	
Output Viral Load (log_{10})	**≥ 7.50 ± 0.00**	**≤ 2.80****		**≥ 7.50 ± 0.00**	**≤ 2.80****	

** These samples serve as the neutralizer effectiveness controls.*
*** The large volume titer was used in the calculation as it was more sensitive.*
[a] The cytopathic appearance in these wells was determined to be virus casued cytopathic effects (CPE)

Table 2 – Controls

(Dilution of the neutralized sample or virus stock (log_{10})	Virus Recovery Control	Column Titer Control**	Virus Stock Titer Control
-2	4/4	4/4	n.d.
-3	4/4	4/4	4/4
-4	4/4	4/4	4/4
-5	4/4	4/4	4/4
-6	4/4	4/4	4/4
-7	2/4	4/4	4/4
-8	n.d.	n.d.	3/4
$TCID_{50}$/mL (log_{10})	7.00 ± 0.28	≥ 7.50 ± 0.00	8.25 ± 0.25
Volume of sample (mL)	10	10	NA
Initial Viral Load (log_{10})	**8.00 ± 0.28**	**≥ 8.50 ± 0.00**	NA

*** This is the Virus Recovery Control sample prior to passing through the gel filtration column.*

RESULTS (continued)

Table 3 – Controls

Dilution of the neutralized sample (log_{10})	Cytotoxicity Control		Viral Interfering Control Virus Titer (log_{10})		
	PWRB001 (2)	PWRB001 (6)	Medium-treated cells	PWRB001 (2) – treated cells	PWRB001 (6) – treated cells
-1	C/4	C/4	8.25 ± 0.25	n.d.	n.d.
-2	C/4	C/4		n.d.	n.d.
-3	0/4	0/4		7.75 ± 0.25	8.25 ± 0.25
Cell Viability Control	0/4				

Table 4 – Viral Reduction Factor(s)

Test Agent	Contact Time	Initial Viral Load (log_{10} $TCID_{50}$)	Output Viral Load (log_{10} $TCID_{50}$)	log_{10} Reduction Factor
PWRB001 (2)	5 min	8.00 ± 0.28	≤ 2.80	≥ 5.20 ± 0.28
PWRB001 (6)	5 min	8.00 ± 0.28	≤ 2.80	≥ 5.20 ± 0.28

CONCLUSIONS

Pluswipes Ltd's test agents PWRB001 (2) and PWRB001 (6) were evaluated for the ability to inactivate Human Influenza A Virus (H1N1). MICROBIOTEST personnel performed the inactivation procedure using Human Influenza A Virus (H1N1) to spike the test agent solutions. Samples were titrated by 50% tissue culture infectious dose per mL ($TCID_{50}$) endpoint assay using MDCK cells.

When tested as described, PWRB001 (2) and PWRB001 (6) inactivated Human Influenza A Virus (H1N1) each by ≥ 5.20 ± 0.28 logs when Human Influenza A Virus (H1N1) was exposed to the test agent(s) for 5 minutes at 21C in the presence of 3.0 g/L BSA + 3.0 mL/L erythrocytes interfering condition (dirty).

Table 4 reports the individual log_{10} virus reduction factor for each of the test agents. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

APPENDIX

001-703-925-9366

MICROBIOTEST PROTOCOL

EN 14476:

VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN HUMAN MEDICINE

Human Influenza A Virus (H1N1)

Prepared for:
Pluswipes Ltd
Pywell Road
Willowbrook East Industrial Estate
Corby, Northants, NN17 5XJ
United Kingdom

August 5, 2009

MICROBIOTEST Protocol: 693.1.08.05.09

MICROBIOTEST Project: 693 - 102

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a product to be labeled as a virucide. It determines the potential of the test agent to inactivate Human Influenza A Virus (H1N1) in suspension. This test conforms to the principles of the European Standard EN 14476:2005 (E) "Chemical disinfectants and antiseptics – Virucidal Quantitative Suspension Test for Chemical Disinfectants and Antiseptics Used in Human Medicine – Test method and requirements (phase 2, step 1)".

TESTING CONDITIONS:

The test agent will be evaluated against the challenge virus in suspension. Two types of test agent, one lot each, will be evaluated at one exposure (contact) time point. One interfering condition will be used. The volume of virus inoculum added to test agent will be kept at 10% of the total volume of the mixture. The test agent will be evaluated at one concentration (use dilution). After the contact time, the sample will be immediately neutralized; serially diluted and inoculated onto an appropriate host cell system to determine the amount of infectious virions. One replicate run will be performed for each test condition. No reference inactivation agent will be tested in this protocol.

MATERIALS:

A. The test agent will be supplied by the sponsor.

The test agent will be tested at concentrations and conditions specified by the sponsor (see last page – miscellaneous information). All operations performed on the test agent such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MICROBIOTEST testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MICROBIOTEST will retain all unused test agents for a period of at least three months after completion of the test, then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MICROBIOTEST, including, but not limited to:

1. Challenge virus (requested by the sponsor of the study): Human Influenza A virus (H1N1), A/PR/8/34

   Note: A window of reduction of ≥ 4.0-log is targeted for this study by using the highest titered virus possible and, if necessary, large volume inoculation.

2. Host: MDCK cells

3. Laboratory equipment and supplies.

   Laboratory equipment and supplies relevant to the performance of the assay will be documented in the data pack.

4. Media and reagents:

   a. Sterile hard water for dilution of products will be prepared as described in EN 14476:2005 (if required)
   b. Dilution medium (DM)
   c. Bovine serum albumin fraction V (BSA), prepared in deionized water (if required)

   Media and reagents relevant to the virus-host system and test agent being tested will be documented in the first project sheet and the data pack.

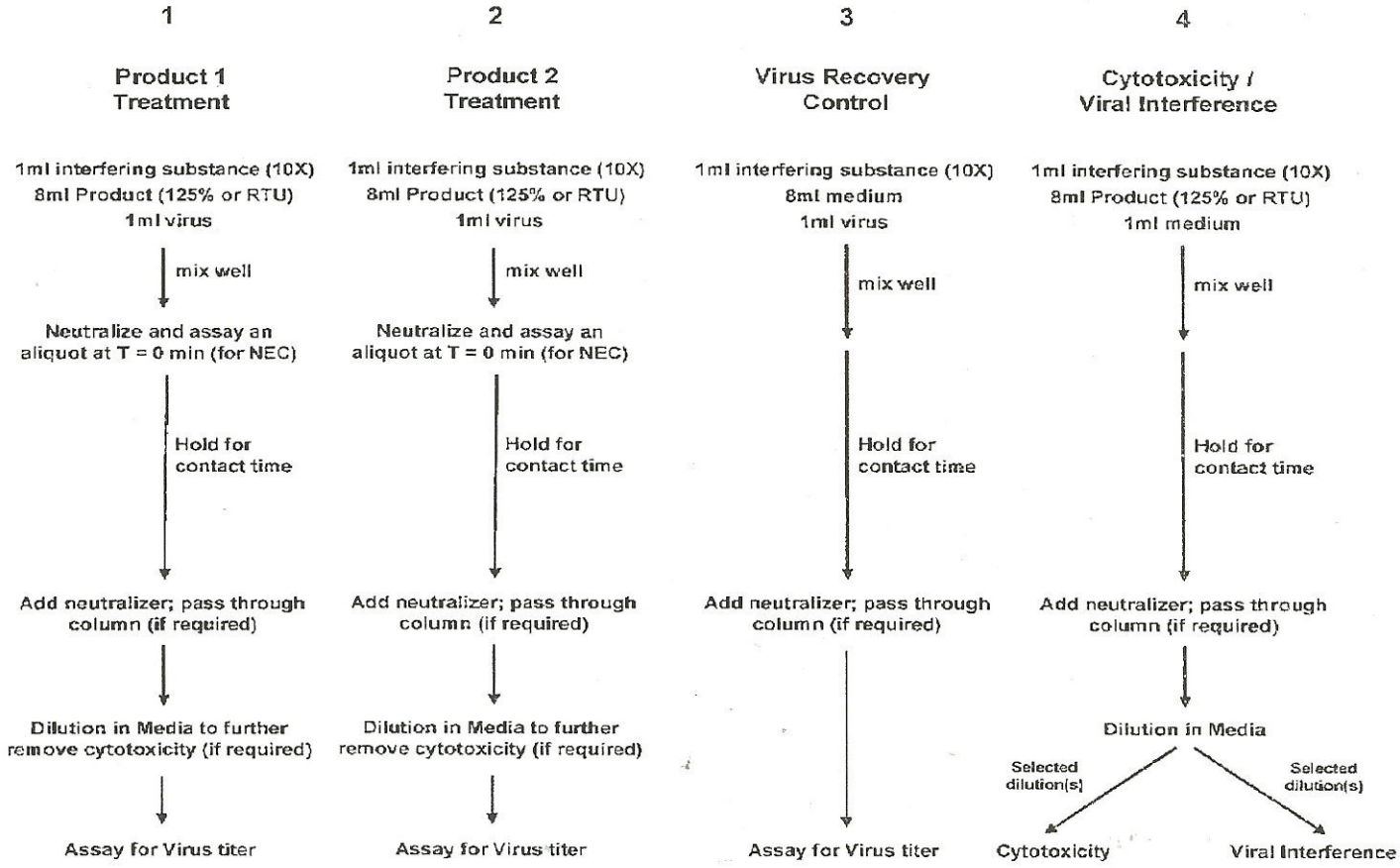
TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be labeled with virus identification and project number.

EXPERIMENTAL DESIGN:

Procedures involved in performance of the study are described in a series of SOPs and logs that are maintained at MICROBIOTEST. All assay reagents (except virus suspension), including test agent, will be equilibrated to the test temperature before testing. The study flow diagram is summarized in Figure 1, with details described in the following sections.

FIGURE 1



NEC: Neutralizer Effectiveness Control

Note 1: One contact time will be evaluated for each test agent.
Note 2: The cytotoxicity and viral interference controls will be performed on each type of test agents.

A. Preparation of interfering substances:

The interfering substances shall be prepared at the 10X the final concentration required for the test (with the exception of sheep erythrocytes). The following interfering condition will be used.

A.1 "Dirty" conditions for instrument and surface disinfectants:

BSA (30 g/L) will be prepared in deionized water and membrane filtered. The final concentration of BSA in the test will be 0.3% (=3 g/L).

Sheep erythrocytes will be washed at least three times in PBS by spinning at 800g, 10 minutes. Three milliliter of packed sheep erythrocytes will be resuspended in 97 mL of 3% w/v (30 g/L) BSA to prepare the 10X interfering substance. The final concentration of sheep erythrocytes in the test will be 3 mL/L.

Note: the interfering substance containing both BSA and sheep erythrocytes will be used in the study.

B. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at MICROBIOTEST. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test (fresh stock cultures may be used at the discretion of the Study Director). No additional serum will be added to the viral inoculum.

C. Test agent preparation:

Two types of test agent will be tested. The test agent will be prepared according to the sponsor's specifications and tested at one concentration (use dilution).

The test agent will be diluted using EN14476 hard water to 125% of the use concentration; or if the test agent is a ready-to-use, the test agent will be tested as

is. The prepared concentration of the test agent is 1.25 times the desired final concentration as the test agent will be diluted to 80% during the test procedure.

The product test solutions will be used within two hours of preparation.

D. Test:

For each type of test agent, one mL of test virus suspension will be mixed with one mL of the 10X interfering substance. Eight ml of the test agent at appropriate concentration will then be added. The reaction mixture will be thoroughly mixed and a stop-watch will be started concurrently.

Immediately after preparation of the test mixture, at time 0, an aliquot of the reaction mixture will be removed, neutralized with an appropriate neutralizer, passed through a gel filtration column (if required), and then diluted ten-fold in dilution medium (DM). The diluted sample will be held under ice bath for 30 min. This will be the Neutralizer Effectiveness Control (see Section F3).

The reminder of the test mixture will be held for the contact time as specified by the sponsor in the last page of the protocol. After contact time, an aliquot of the reaction mixture will be removed, neutralized with neutralizer, passed through a gel filtration column (if required), and ten-fold serially diluted in DM. Prior to the serial dilution, the neutralized sample may be diluted with DM to further remove cytotoxicity. The sample will then be serially ten fold diluted and inoculated onto host cells to assay for infectious virus.

If columns are used to reduce toxicity, an aliquot of each reaction mixture will be loaded into separate pre-spun Sephacryl columns. Following passage through columns, the eluate will be collected aseptically and diluted with DM, as described above.

E. Infectivity assay:

The residual infectious virus in both test and controls will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the neutralized inoculum/disinfectant mixture will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2C with 5±1% CO_2 for a period of 4-6 days. The host cells may

be washed twice with phosphate buffered saline (PBS) prior to inoculation. The host cell cultures will be observed and refed as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific CPE and test agent-specific cytotoxic effects will be scored by examining both test and controls. These observations will be recorded.

In order to increase the sensitivity of the viral infection assay, a large volume sampling of the test agent-treated sample(s) may be performed by inoculating the sample at the lowest dilution without significant cytotoxicity or viral interference onto a large number of replicate wells of the host cells.

F. Controls:

1. Virus Recovery Control:

To check for the infectivity of the test virus suspension under the test conditions, the stock virus will be mixed with the interfering substance and cell culture medium for the contact time specified by the sponsor as described above for the test agent. The test mixture will be prepared by mixing 1 mL stock virus with 1 mL10X interfering substance, and 8 mL cell culture medium (in lieu of the test agent). This control will determine the virus recovery and relative loss in virus infectivity resulting from holding alone.

2. Cytotoxicity and Viral Interference Control:

The cytotoxicity and viral interference control will be performed for each type of test agents.

To check for possible morphological alteration/destruction of cells by the test agent, a 8-mL aliquot of test agent (125% of the target use concentration or the undiluted product if it is ready to use) will be mixed with 1 mL 10X interference substance and 1 mL medium and held for the specified contact time. At the end of the contact time, the reaction mixture will be neutralized with an appropriate neutralizer and, if required, passed through a Sephacryl column (to further reduce cytotoxicity). The sample will then be serially diluted with DM and selected dilutions will be assessed for cytotoxicity and viral interference as described below.

For cytotoxicity assessment, an appropriate amount of each dilution of the neutralized sample will be inoculated onto the cell monolayer and incubated as described above. After incubation, the cell monolayer will be microscopically examined for morphology change.

For viral interference assessment, cell monolayers will be treated with an appropriate amount (0.2 mL per well) of either a selected dilution of the neutralized test agent or the cell culture medium for one hour at 36±2C. After the incubation, the supernatant will be discarded. The stock virus will then be titrated in parallel on both types of treated cell monolayers to obtain a titer.

Only those dilutions of the neutralized product solution that (a) show a low degree of cell destruction (< 25% of monolayer); and (b) produce a titer reduction of the virus of < 1 log can be used for the determination of the residual infectivity.

3. Neutralizer Effectiveness Control (NEC):

This control will be performed for each type of test agents. It will determine if residual active ingredient is present after addition of neutralizer. Immediately after preparation of the test mixture (section D), at time 0, an aliquot of the reaction mixture will be removed, neutralized, passed through column (if required), and ten-fold diluted in DM. The diluted sample will be held under ice bath for 30 min. After incubation, the samples will be inoculated onto the host cells for virus infectivity determination as described above.

The difference of titer between the NEC and VRC samples shall be ≤ 0.5 $\log_{10}$.

4. Cell viability control:

This control will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the CCM employed throughout the assay period.

An appropriate amount of medium will be added to at least four wells of the indicator cells and incubated together with other test and control samples. This will serve as the negative control.

5. Virus Stock Titer control (VST)

An aliquot of the challenging virus used will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

G. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the Spearman-Karber method. In the case where a sample contains no detectable virus, a statistical analysis may be performed based on Poisson distribution to determine the theoretical maximum possible titer for that sample. These analyses will be described in detail in the final report. The test results will be reported as the reduction of the virus titer due to treatment with test agent expressed as $\log_{10}$.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The test virus suspension has $TCID_{50}$ 10^8/mL or minimally a concentration that exceeds the toxicity endpoint by ≥ 4-$\log_{10}$
- The cytotoxicity of the product solution does not affect host cell viability in the dilutions of the test mixtures which are necessary to demonstrate a 4.0-log reduction of the virus
- Viral-induced cytopathic effect (CPE) is distinguishable from test agent induced cytotoxic effect
- Virus is not detected in the cell viability control
- The difference of titer between the NEC and VRC samples is ≤ 0.5 $\log_{10}$
- The difference of titer between the neutralized product-treated and cell culture medium-treated monolayers is ≤ 1 $\log_{10}$

PRODUCT EVALUATION CRITERIA:

According to the regulatory agencies, the test agent passes the test if there is at least a 4.0-log reduction in titer beyond the cytotoxicity level.

REPORT FORMAT:

MICROBIOTEST employs a standard report format for each test design. Each final report will provide the following information:

- Sponsor identification
- Test agent identification
- Type of assay and project number
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge virus and host used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs project sheet number one will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study:

A. Name and address: Pluswipes Ltd
Pywell Road
Willowbrook East Industrial Estate
Corby, Northants, NN17 5XJ
United Kingdom

B. Test agent 1: ☑ PWRB001 (2) ~~Response Beta Antiseptic~~
Active ingredient: ☑ Polyaminopropyl Biguanide, Benzalkonium Chloride
Lot No: ☑ FF 590/2
Product use dilution: ☑ Ready to use

Test agent 2: ☑ PWRB001 (6) ~~Response Beta Antiseptic~~
Active ingredient: ☑ Polyaminopropyl Biguanide, Benzalkonium Chloride
Lot No: ☑ FF 659/1
Product use dilution: ☑ Ready to use

Diluent for test agent: ☐ Not applicable

Interfering condition (check one):
☑ 3.0 g/L BSA + 3.0 mL/L erythrocytes final ("dirty")

Exposure (Contact) time: ☑ 5 minutes
Exposure temperature: ☑ 20C ± 1 C

C. Precautions/storage conditions: refer to MSDS or certificate of analysis
☐ provided ☑ ~~not~~ provided

REPORT HANDLING:

The sponsor intends to submit this information to (CHOOSE ONE):

☐ EPA ☐ FDA ☐ Health Canada ☐ CAL DPR
☐ ARTG ☐ non GLP ☑ ~~other~~ _____

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 6/08/09

Printed Name: M. TEASDALE-BROWN

MicroBioTest, Inc., 105B Ca ıter Dr., Sterling, Virginia 20164

Date Issued: 08/10/09 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 693-102

STUDY TITLE: EN 14476: VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN HUMAN MEDICINE Human Influenza A virus (H1N1)	**STUDY DIRECTOR:** S. Steve Zhou, Ph.D. Signature 08/11/09 Date		
TEST MATERIAL(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
PWRB001 (2)	FF 590/2	7/6/09	10169
PWRB001 (6)	FF 659/1	7/6/09	10170
PERFORMING DEPARTMENT(S): Virology Department	**STORAGE CONDITIONS:** Location: C2 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		

PROTECTIVE PRECAUTION REQUIRED: MSDS ☐ Yes / ■ No

PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol ☐ Other:

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 08/11/09 **TERMINATION DATE:** 08/18/09

CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: European Agencies

SPONSOR: Plus Wipes, Ltd. Pywell Road Willowbrook East Industrial Estate Corby, Northants, NN17 5XJ United Kingdom	**CONTACT PERSON: Marten Teasdale Brown** Telephone No. +44 (0) 1536 446150 FAX No. +44 (0) 1536 408789

TEST CONDITIONS:

Challenge organism(s):	Human Influenza A Virus (H1N1), A/PR/8/34, Charles River Laboratories
Host:	MDCK cells, ATCC CCL-34
Dilution medium:	MEM + 1 µg/mL Trypsin
Active ingredient(s):	Polyaminopropyl Biguanide, Benzalkonium Chloride
Neutralizer(s):	MEM + 1% Fetal bovine serum + 0.5% Lecithin
Contact time(s):	5 minutes Contact temperature(s): 20±1C
Dilution(s):	Ready to use
Interfering condition:	3.0 g/L BSA + 3.0 mL/L erythrocytes (dirty)
Incubation time(s):	4-6 days Incubation temperature(s): 36±2C with 5±1% CO_2
Comment:	The active ingredients will not be listed in the final report per sponsor's request

MicroBioTest, Inc., 105B Carpenter Dr., Sterling, Virginia 20164

Date Issued: 08/26/09 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 693-102

STUDY TITLE: EN 14476: VIRUCIDAL QUANTITATIVE SUSPENSION TEST FOR CHEMICAL DISINFECTANTS AND ANTISEPTICS USED IN HUMAN MEDICINE Human Influenza A virus (H1N1)	**STUDY DIRECTOR:** S. Steve Zhou, Ph.D. Signature 08/26/09 Date		
TEST MATERIAL(S):	**LOT NO.**	**DATE RECEIVED:**	**DS NO.**
PWRB001 (2)	FF 590/2	7/6/09	10169
PWRB001 (6)	FF 659/1	7/6/09	10170
PERFORMING DEPARTMENT(S): Virology Department	**STORAGE CONDITIONS:** Location: C2 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: European Agencies			
SPONSOR: Pluswipes Ltd Pywell Road Willowbrook East Industrial Estate Corby, Northants, NN17 5XJ United Kingdom	**CONTACT PERSON: Marten Teasdale Brown** Telephone No. +44 (0) 1536 446150 FAX No. +44 (0) 1536 408789		

EXPLANATION:

This project sheet was issued to document the following:

Protocol Amendment(s):

1. A Column Titer Control (CTC) was performed to determine any affects of Sephacryl columns on infectious virus titer while passing through the columns. The sample for this control was acquired from a portion of the Virus Recovery Control prior to passing through the column. The sample was used to make ten-fold serial dilutions in cell culture medium (CCM). It was then processed in the same manner as the test. This amendment serves to define the CTC performed in the test that was not mentioned in the protocol.

2. Project Sheet No. 1 states that the proposed experimental start date was 08/11/09. The actual experimental start date was 08/14/09. Additionally, the sponsor name should be Pluswipes Ltd. Instead of Plus Wipes, Ltd. This amendment serves to correct the typographical error in the Project Sheet No. 1.