MICROBAC®

FINAL REPORT

Evaluation of Viral Elimination from a Solid Surface Material by Bleach or Alcohol – Human Immunodeficiency Virus Type 1 (HIV-1)

TEST ARTICLE
Diluted Bleach
70% Alcohol

TEST SURFACE
HI-MACS®

Author
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Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
982-101

Sponsor
LG Hausys Ltd.
One IFC 10 Gookjegeumyoong-Ro,
Yeongdeungpo-Gu
Seoul, 150-876, Korea

TABLE OF CONTENTS

COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR § 160.

The following technical personnel participated in this study:

Tanya Kapes, Jennifer Purgill, and Cameron Wilde

Study Director: Microbac

Tanya Kapes — Date: 1/8/19

QUALITY ASSURANCE UNIT STATEMENT

Title: Evaluation of Viral Elimination from a Solid Surface Material by Bleach or Alcohol – Human Immunodeficiency Virus Type 1 (HIV-1)

The Quality Assurance Unit of Microbac has inspected Project Number 982-101 in compliance with current Good Laboratory Practice regulations (40 CFR § 160).

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	12/06/18, 12/07/18	12/07/18	12/07/18
In-Process	12/07/18	12/07/18	12/07/18
Final Report	12/31/18	01/02/19	01/02/19

Danielle Downs, RQAP-GLP
Quality Assurance Specialist III — Date: 01/08/19

TEST SUMMARY

TITLE: Evaluation of Viral Elimination from a Solid Surface Material by Bleach or Alcohol – Human Immunodeficiency Virus Type 1 (HIV-1)

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

TEST ARTICLES: Diluted bleach (0.5% sodium hypochlorite), prepared 12/07/18 using Clorox Bleach, Lot No. E618309 MD2311 and Sterile Deionized Water, NL No. 1218-18; assigned DS No. I681a

70% Isopropanol (IPA), prepared on 12/07/18, NL No. 1220-18; assigned DS No. I681b

TEST SURFACES:

TEST SURFACE NAME	LOT NO.	RECEIVED DATE	ASSIGNED DS NO.
HI-MACS®	3854	11/29/18	I681

SPONSOR: LG Hausys Ltd.
One IFC 10 Gookjegeumyoong-Ro,
Yeongdeungpo-Gu
Seoul, 150-876, Korea

TEST CONDITIONS

Challenge virus:

Human Immunodeficiency Virus Type 1, strain: IIIB, source: ZeptoMetrix

Host:

C8166 cells, source: University of Pennsylvania

Disinfectant treatment procedure:

Each contaminated test surface was submerged in disinfectant and held for 30 seconds. Excess disinfectant was drained and the surface was then submerged in Sterile Deionized Water for 30 seconds and drained in a similar fashion. Test surfaces were placed in new sterile petri dishes and 4 mL of neutralizer was added. Test surfaces were scraped and the neutralized mixture's pH was found to be 8.0 for all disinfectant runs. The neutralized mixture was diluted 10-fold and plated on host cells.

Test Article	Active Ingredient(s)	Contact Time	Neutralizer
Diluted Bleach	0.5% sodium hypochlorite	30 seconds per submersion	RPMI 1640 + 10% Fetal Bovine Serum + 0.5% $Na_2S_2O_3$
70% Alcohol	70% Isopropanol	30 seconds per submersion	RPMI 1640 + 10% Fetal Bovine Serum

Dilution medium:

RPMI 1640 + 2% Fetal Bovine Serum (FBS)

Carrier preparation, inoculation, and dry time:

The test surfaces were steam sterilized for 15 minutes at 121°C, cooled and stored at room temperature prior to testing. Surfaces were UV irradiated for ≥15 minutes per side, then were inoculated with 0.4 mL of virus and dried for 40 minutes at 20°C.

Contact temperature:

Ambient room temperature 20±2°C (Actual: 20°C)

Organic load:

Virus contained 5% serum

Incubation temperature:

36±2°C in 5±3% CO_2

TEST CONDITIONS (continued)

Media and reagents:

RPMI 1640 + 2% FBS
RPMI 1640 + 10% FBS
RPMI 1640 + 10% FBS + 0.5% $Na_2S_2O_3$
Sterile Deionized Water
pH paper
RPMI 1640 + 5% FBS

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164. Testing was initiated on 12/07/18 and was completed on 12/19/18. The study director signed the protocol on 12/07/18. 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 article records, the final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 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 dilution at which half of the wells are infected 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
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 0.05 mL.

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

$$\sigma_m = d_f \sqrt{\sum \frac{p_i(1-pi)}{(ni-1)}}$$

where:

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

and $\sum$ denotes the summation over dilutions beginning at the k^{th} dilution. The 95% confidence interval is $m \pm 1.963\sigma_m/2$.

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

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.

CALCULATION OF VIRAL REDUCTION FACTOR

The results are reported as the reduction of the virus load due to process step expressed as $\log_{10}$, calculated based on the guidelines of CPMP/BWP/268/95, "Note for Guidance on virus Validation Studies: The Design, Contribution and interpretation of Studies validating the Inactivation and Removal of Viruses".

<u>The formula for determining the log reduction factor (LRF) for each step is:</u>

$$\text{LRF} = \text{Log}_{10} \left(\frac{\text{Input Titer x Input Volume}}{\text{Output Titer x Output Volume}} \right)$$

CALCULATION OF VIRAL REDUCTION FACTOR (continued)

When a sample is diluted and/or neutralized prior to being assayed, a volume correction factor should be included in the calculation of the viral load.

Viral Load (log_{10}) = Virus Titer (log_{10}/mL) + log_{10} (volume x volume correction)

The Average Viral Log_{10} Load from n replicates was determined as follows:

$$Log_{10}\left[\frac{10^{(Log_{10}Load_1)} + 10^{(Log_{10}Load_2)} + \ldots + 10^{(Log_{10}Load_n)}}{n}\right]$$

The 95% Confidence Limits (CL) for the LRF are calculated as follows:

$$(CL_{LRF})^2 = (CL_{input})^2 + (CL_{output})^2$$

In the case when all negatives are observed, replace the output load by c x Output Volume for calculating the log reduction, where c is taken from the Poisson 95% confidence limit discussed above, and substitute 0 for CL_{output} in calculating the 95% confidence limits of the log reduction factor.

RESULTS

Data are presented in Tables 1 – 3.

Table 1
Titer results

Surface	Treatment	Replicate	Titer ± 95% CL ($Log_{10}TCID_{50}$/mL)	Volume (mL) [A]	Viral Load ($Log_{10}TCID_{50}$)
HI-MACS®	**Diluted Bleach** (30 second submersion) + Rinse (30 second submersion)	Rep 1	≤ 0.83 *	4.0	≤ 1.43
		Rep 2	≤ 0.83 *		≤ 1.43
		Rep 3	≤ 0.83 *		≤ 1.43
	70% IPA (30 second submersion) + Rinse (30 second submersion)	Rep 1	≤ 0.83 *	4.0	≤ 1.43
		Rep 2	≤ 0.83 *		≤ 1.43
		Rep 3	≤ 0.83 *		≤ 1.43
	Rinse only (30 second submersion)	Rep 1	4.93 ± 0.12	4.0	5.53 ± 0.12
		Rep 2	4.93 ± 0.12		5.53 ± 0.12
		Rep 3	4.93 ± 0.24		5.53 ± 0.24
	Untreated	Rep 1	5.30 ± 0.19	4.0	5.90 ± 0.19
		Rep 2	5.55 ± 0.25		6.15 ± 0.25
		Rep 3	5.55 ± 0.16		6.15 ± 0.16
		Average Viral Load			6.08 ± 0.20

[A] Volume refers to the volume of the virus recovery solution.

* No virus was detected; the theoretical titer was determined based on the Poisson distribution.

Table 2
Controls

Sample	Results
Neutralization/Viral Interference – Diluted Bleach	Virus detected in all wells
Cytotoxicity Control – Diluted Bleach	no cytotoxicity observed
Neutralization/Viral Interference – 70% IPA	Virus detected in all wells
Cytotoxicity Control – 70% IPA	no cytotoxicity observed
Cell Viability Control	no virus detected, cells were viable; media was sterile
Virus Stock Titer Control	6.55 ± 0.16 Log_{10} Titer ($TCID_{50}$/mL)

RESULTS (continued)

Table 3
Reduction factors

Surface	Treatment	Input Viral Load ($Log_{10}TCID_{50}$)[B]	Replicate	Output Viral Load ($Log_{10}TCID_{50}$)	Reduction ($Log_{10}TCID_{50}$)
HI-MACS®	**Diluted Bleach** (30 second submersion) + Rinse (30 second submersion)	6.08 ± 0.20	Rep 1	≤ 1.43	≥ 4.65 ± 0.20
			Rep 2	≤ 1.43	≥ 4.65 ± 0.20
			Rep 3	≤ 1.43	≥ 4.65 ± 0.20
	70% IPA (30 second submersion) + Rinse (30 second submersion)	6.08 ± 0.20	Rep 1	≤ 1.43	≥ 4.65 ± 0.20
			Rep 2	≤ 1.43	≥ 4.65 ± 0.20
			Rep 3	≤ 1.43	≥ 4.65 ± 0.20
	Rinse only (30 second submersion)	6.08 ± 0.20	Rep 1	5.53 ± 0.12	0.55 ± 0.23
			Rep 2	5.53 ± 0.12	0.55 ± 0.23
			Rep 3	5.53 ± 0.24	0.55 ± 0.31

[B] Input Viral Load is the average Viral Load of the untreated samples.

CONCLUSIONS

A treatment procedure of a 30 second submersion in diluted bleach (5,000 ppm sodium hypochlorite) followed by a 30 second submersion in Sterile Deionized Water was able to completely inactivate HIV-1 (≥4.65 Log_{10} reduction) from the HI-MACS® material.

A treatment procedure of a 30 second submersion in 70% Isopropanol followed by a 30 second submersion in Sterile Deionized Water was able to completely inactivate HIV-1 (≥4.65 Log_{10} reduction) from the HI-MACS® material.

A treatment procedure of a 30 second submersion in Sterile Deionized Water alone reduced the viral load of HIV-1 from the HI-MACS® material by 0.55 Log_{10}.

All of the controls met the criteria for a valid test. These conclusions are based on observed data.

APPENDIX I

MICROBAC®

Microbac Protocol

Evaluation of Viral Elimination from a Solid Surface Material by Bleach or Alcohol –

Human Immunodeficiency Virus Type 1 (HIV-1)

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
LG Hausys America, Inc.
900 Circle 75 Pkwy #1500
Atlanta, GA 30339

November 20, 2018

Microbac Protocol: LGH.1.11.20.18

Microbac Project: 982-101

Microbac Laboratories, Inc.
105 Carpenter Drive | Sterling, VA 20164 | 703.925.0100 p | 703.925.9366 f | www.microbac.com

OBJECTIVE:

This test is designed to evaluate the viral elimination effectiveness of bleach or alcohol from a hard surface material against the target virus. The test conforms in principle to U.S. EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, and follows the procedure outlined in the ASTM International test method designated E1053-11, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces".

One target virus – Human Immunodeficiency Virus (HIV-1) - will be tested in this protocol.

TESTING CONDITIONS:

Two disinfectants (diluted bleach and 70% alcohol) will be evaluated on one type of surface material at three replicates (N=3) per condition. In addition, a recovery control without any treatment and a rinsing control will be performed using the same level of viral challenge on each type of surface material.

For each run, a test surface material ("carrier") will be inoculated with 0.4-mL of HIV-1 fluid, dried, treated with the disinfectant by completely submerging the carrier into the disinfectant (diluted bleach or 70% alcohol) for 30 seconds, followed by rinsing via submersion in sterile deionized water for 30 seconds. After rinsing, the coupon will be placed in a sterile petri dish and 4 mL of the recovery medium (= neutralizer) will be added onto the carrier and then the viral inoculum will be scraped off from the carrier using a cell scraper into the neutralizer. The carrier-eluted sample will be neutralized to pH 6 – 8 if the sample is not within this pH range.

The sample will be serially diluted in DM and selected dilutions will be inoculated onto host cells to determine the titer of infectious virus. The titer will be compared to a carrier recovery control where the same amount of virus is added to the carrier, dried, untreated, and recovered by the same neutralizer, to determine the Log_{10} reduction value (LRV) by the disinfectant.

MATERIALS:

A. Test materials will be supplied by the sponsor of the study (see last page).

The test material will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test material such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

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

Microbac will retain all unused test materials for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer.

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

1. Challenge virus (requested by the sponsor of the study): Human Immunodeficiency Virus Type 1 (HIV-1), strain: IIIB

2. Host cell line: C8166 cells

3. Laboratory equipment and supplies

4. Disinfectants:
   - Diluted bleach (final concentration: 0.5% sodium hypochlorite)
   - 70% Isopropanol

5. Media and reagents, including but not limited to:
   - Virus recovery medium (= neutralizer)
   - Sterile deionized (DI) water
   - Cell culture medium
   - Dilution medium (DM)

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

TEST SYSTEM IDENTIFICATION:

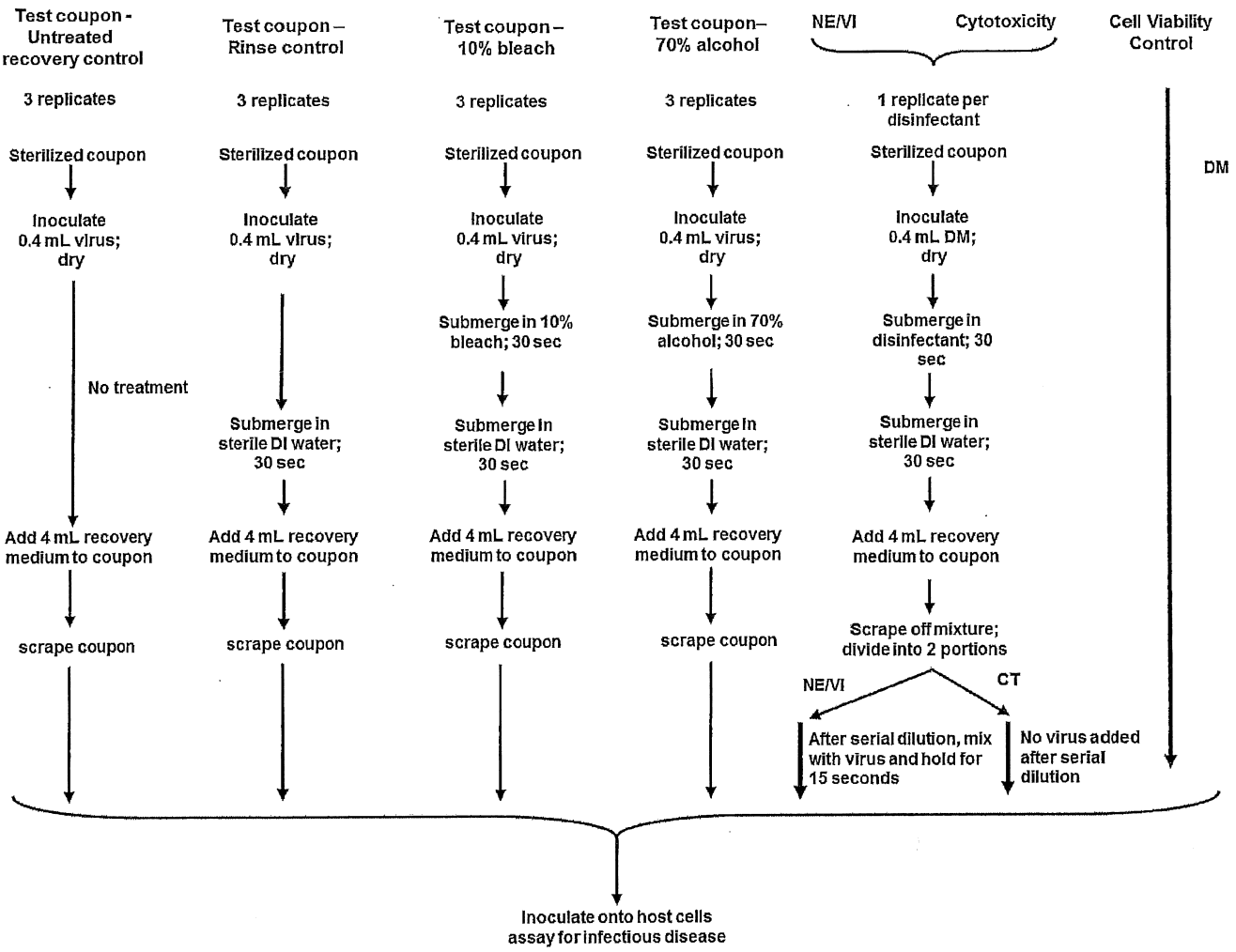
All Petri dishes, dilution tube racks, and host-containing apparatus will be labeled with virus identification and project number.

EXPERIMENTAL DESIGN:

The procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac SOPs and Logs are referred to in the raw data and are required as part of GLP regulations.

The study flow diagram is summarized in Figure 1.

Figure 1
Study Flow Diagram



Note: one type of surface material will be evaluated at three replicates (N=3) per condition. NE/VI and Cytotoxicity will be performed for both disinfectants.

A. Viral inoculum preparation:

Viral stocks are obtained from reputable sources that identify them by scientifically accepted methods and may have been propagated at Microbac. 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 (freshly prepared viral stocks may also be used). The organic soil concentration will be adjusted to 5% (if not already 5%) for the virus stock unless otherwise directed by the Sponsor and pre-approved by Microbac.

B. Coupon (Carrier) preparation:

The carriers will be sterilized by one of the following techniques as appropriate depending on the type of surface:

- Place the coupons in an autoclave bag and steam sterilize for at least 10 minutes at 121°C; cool and store them at room temperature until use. Immediately before use, expose the coupons to Ultra-Violet (UV) light for at least 15 minutes per side.

- For coupons that cannot tolerate autoclave, place them in an evaporating dish matted with filter paper and heat them in a hot air oven for two hours at 180°C; cool and store them at room temperature until use. Immediately before use, expose the coupons to Ultra-Violet (UV) light for at least 15 minutes per side.

The exact sterilization procedure utilized will be described in the final report.

C. Disinfectant preparation:

Each disinfectant will be prepared based on the preparation instructions provided by either the manufacturer or the sponsor.

- The diluted bleach will be prepared immediately prior to testing from concentrated bleach diluted in sterile deionized water to a final concentration of 0.5% Sodium Hypochlorite.

- 70% Alcohol (Isopropanol) will either be prepared prior to testing by diluting 2-propanol in sterile deionized water or will be obtained ready to use from a suitable source.

D. Carrier inoculation:

One type of surface material will be evaluated. Each coupon has an approximate 2" x 2" area.

An aliquot of 0.4 mL of stock virus will be added to each carrier and spread with a cell scraper over the surface of the coupon and allowed to dry at room temperature (usually 20 – 30 minutes). The drying time and temperature will be recorded.

Three carriers (N=3) will be prepared for each test surface and each test condition (see Figure 1 for details). These carriers will then be exposed to the disinfectants as described below.

Additionally, three carriers (N=3) will be prepared for the Recovery Control for each test surface. These carriers will be processed without any treatment.

E. Virucidal efficacy evaluation

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac. SOPs and Logs are referred to in the raw data. The performance and sample collection for the study are diagrammed in Figure 1, with details described below.

One type of surface material will be evaluated at three replicates (N=3) per condition.

1. Disinfectant treatment procedure – diluted bleach:

For each run, after the virus inoculum has dried, coupons will be handled with sterile thumb forceps and dipped for 30 seconds in diluted bleach (0.5% sodium hypochlorite). Excess will be allowed to drain back into the disinfectant container. The coupon will then be dipped in sterile DI water for 30 seconds and drained in a similar fashion. The coupon will then be placed in a new sterile petri dish and 4 mL of recovery medium shall be added. The

coupon shall be scrapped with a cell scraper and the neutralized mixture will be measured for pH using pH paper. If the pH is not within pH 6 – 8, the sample will be neutralized to pH 6 – 8 using an appropriate substance (i.e. HCl or NaOH). This manipulation will be recorded and reported.

This sample, considered Undilute (10^0), will be serially diluted in DM and selected dilutions will be inoculated onto host cells to determine the titer of infectious virus as described in Section G.

2. Disinfectant treatment procedure – 70% isopropanol:

For each run, after the virus inoculum has dried, coupons will be handled with sterile thumb forceps and dipped for 30 seconds in 70% isopropanol. Excess will be allowed to drain back into the disinfectant container. The coupon will then be dipped in sterile DI water for 30 seconds and drained in a similar fashion. The coupon will then be placed in a new sterile petri dish and 4 mL of recovery medium shall be added. The coupon shall be scrapped with a cell scraper and the neutralized mixture will be measured for pH using pH paper. If the pH is not within pH 6 – 8, the sample will be neutralized to pH 6 – 8 using an appropriate substance (i.e. HCl or NaOH). This manipulation will be recorded and reported.

This sample, considered Undilute (10^0), will be serially diluted in DM and selected dilutions will be inoculated onto host cells to determine the titer of infectious virus as described in Section G.

3. Control treatment procedure – rinse only:

For each run, after the virus inoculum has dried, coupons will be handled with sterile thumb forceps and dipped for 30 seconds in sterile deionized water. Excess will be allowed to drain back into the container. The coupon will then be placed in a new sterile petri dish and 4 mL of recovery medium shall be added. The coupon shall be scrapped with a cell scraper and the neutralized mixture will be measured for pH using pH paper.

This sample, considered Undilute (10^0), will be serially diluted in DM and selected dilutions will be inoculated onto host cells to determine the titer of infectious virus as described in Section G.

F. Controls

1. Untreated recovery control:

For each run, the same amount of virus inoculum as used in the test substance runs will be applied onto the materials and let dry at ambient temperature. No treatment will be applied. 4.0 mL of neutralizer will be added to recover the inoculum. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The results from this control will be compared with the test-agent results to calculate the log reduction value (LRV) of the challenge virus.

2. Neutralizer effectiveness/viral interference control:

This control will be performed on each disinfectant procedure at one replicate. It will determine if residual active ingredient is present after neutralization and if the neutralized test agent interferes with the viral infectivity assay.

This control will be processed exactly as the test procedure but in lieu of the viral inoculum, 0.4 mL of media will be dried onto the coupon and then treated in the same manner as the test. Post procedure and neutralization, the 4.0 mL sample will be divided into two portions, one for cytotoxicity control and the other for neutralizer effectiveness/viral interference control and processed as the test.

For the Neutralizer effectiveness/viral interference control, following the serial dilution, 100 µL of low-titer virus (containing no more than 5,000 units of virus) will be added to 4.5 mL of each selected dilution and held for a period not less than the contact time. Then these samples will be used to inoculate host cells as described for the test procedure.

3. Cytotoxicity control

This control will be performed on each disinfectant procedure at one replicate. The cytotoxicity sample, acquired from the neutralizer effectiveness control, will be diluted and have no virus added. Selected dilutions will be inoculated and incubated in the same manner as the rest of

the test and control samples. The cytotoxic effects, if present, must be distinct from any viral specific cytopathic effects (CPE), which will be evident in the stock titer and virus recovery control cultures.

4. Cell Viability Control:

At least four wells will be inoculated with an appropriate medium during the incubation phase of the study.

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

5. Virus Stock Titer Control (VST):

An aliquot of the virus inoculum used in the study 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. Infectivity assay:

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

Selected dilutions of the neutralized inoculum/test substance mixture (test samples) and control samples will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2°C with 5±3% CO_2 for total 9 – 12 days. The host cells may be washed twice with phosphate buffered saline prior to inoculation. The inoculated culture will be observed and refed with fresh media 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 virus. The resulting virus-specific CPE and test substance-specific cytotoxic effects will be scored by examining all test and control samples. These observations will be recorded.

H. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber. In the case where a sample contains no detectable virus, a statistical analysis will 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. No other statistical analysis will be performed for this study.

The results will be reported as the reduction of the viral load (i.e. $TCID_{50}$ units) by the procedures used.

The formula for determining the log reduction factor (LRF) for each step is:

$$LRF = Log_{10}\left[\frac{\text{Input Titer x Input Volume}}{\text{Output Titer x Output Volume}}\right]$$

When a sample is diluted and/or neutralized prior to being assayed, a volume correction factor should be included in the calculation of the viral load.

Viral Load (log_{10}) = Virus Titer (log_{10}/mL) + log_{10} (volume x volume correction)

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 infectious virus recovered from the Untreated Recovery Control must be $\geq$ 4.8-$\log_{10}$ $TCID_{50}$ units.
- Viral-induced cytopathic effect (CPE) must be distinguishable from test substance induced cytotoxic effects (if any).
- Virus must be recovered from the neutralizer effectiveness/viral interference control (not exhibiting cytotoxicity).
- The Cell Viability Control (assay negative control) must not exhibit virus.

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 Microbac, 105 Carpenter Drive, Sterling, Virginia 20164.

REPORT FORMAT:

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

- Sponsor identification
- Test material 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)

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test material records, final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac, 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 material; 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 the protocol will be the study initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

Table 1

Summary of samples to be assayed (1 of 2)

Sample #	Test Surface	Treatment	Sample description
1	Surface Material	Diluted bleach + Rinse	Surface, 10% bleach, Rep. 1
2			Surface, 10% bleach, Rep. 2
3			Surface, 10% bleach, Rep. 3
4		70% Isopropanol + Rinse	Surface, 70% alcohol, Rep. 1
5			Surface, 70% alcohol, Rep. 2
6			Surface, 70% alcohol, Rep. 3
7		Rinse Only	Surface, Rinse only, Rep. 1
8			Surface, Rinse only, Rep. 2
9			Surface, Rinse only, Rep. 3
10		No treatment	Surface, No treatment, Rep. 1
11			Surface, No treatment, Rep. 2
12			Surface, No treatment, Rep. 3
13		Diluted bleach + Rinse	Surface, 10% bleach, NE/VI control
14			Surface, 10% bleach, Tox control
15		70% Isopropanol + Rinse	Surface, 70% alcohol, NE/VI control
16			Surface, 70% alcohol, Tox control
17	N/A	N/A	Cell Viability Control
18	N/A	N/A	Virus Stock Titer control

NE/VI control: Neutralizer Effectiveness/Viral Interference control
TOX control: Cytotoxicity control

MISCELLANEOUS INFORMATION:

The following is to be completed by Sponsor *(please check all open boxes as applicable)*:

A. Name and address: LG Hausys America, Inc.
900 Circle 75 Pkwy #1500
Atlanta, GA 30339

B. Test Surface name: HI-MACS®

Lot No(s).: _____

C. Disinfectants:

Disinfectant	Active Ingredient(s)	Lot No.
Diluted bleach	0.5% sodium hypochlorite	To be recorded
70% alcohol	70% isopropanol	To be recorded

Contact temperature: Ambient room temperature (20±2°C)

REPORT HANDLING:

The sponsor intends to submit this information to: _____

Study conduct: ☐ GLP

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: _____ Date: 12/5/2018

Printed Name: Gene Lee

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 12/7/18

Printed Name: Tanya Kapes

Date Issued: 12/07/18 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 982-101

STUDY TITLE: Evaluation of Viral Elimination from a Solid Surface Material by Bleach or Alcohol – Human Immunodeficiency Virus Type 1 (HIV-1)	**STUDY DIRECTOR:** Tanya Kapes Tanya Kapes 12/7/18 Signature Date		
TEST MATERIAL(S): HI-MACS®	**LOT NO.** 3854	**DATE RECEIVED:** 11/29/18	**DS NO.** I681
DISINFECTANT(S): Diluted bleach 70% Isopropanol	 TBD TBD	 TBD TBD	 NA NA
PERFORMING DEPARTMENT(S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: K2 ☐ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		

PROTECTIVE PRECAUTION REQUIRED: MSDS/SDS ☐ Yes / ■ No

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

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

PROPOSED EXPERIMENTAL START DATE: 12/07/18 **TERMINATION DATE:** 12/21/18

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

SPONSOR: LG Hausys Ltd. One IFC 10 Gookjegeumyoong-Ro, Yeongdeungpo-Gu Seoul, 150-876, Korea	**CONTACT PERSON:** Gene Lee E-mail: genelee@lghausys.com

TEST CONDITIONS:

Challenge organism(s):	Human Immunodeficiency Virus Type 1 (HIV-1), strain: IIIB, source: ZeptoMetrix
Host cell line:	C8166 cells, source: University of Pennsylvania
Active ingredient(s):	0.5% sodium hypochlorite; 70% Isopropanol
Neutralizer(s):	RPMI 1640 + 10% Fetal Bovine Serum (FBS) [for 70% Isopropanol] RPMI 1640 + 10% FBS + 0.5% $Na_2S_2O_3$ [for diluted bleach]
Dilution Medium:	RPMI 1640 + 2% FBS
Contact Time(s):	30 seconds per submersion
Contact Temperature(s):	Ambient room temperature (20±2°C)
Organic Load:	■ Yes / ☐ No (5% serum in viral inoculum)
Incubation Time(s):	9 – 12 days
Incubation Temperature(s):	36±2°C, 5±3%CO_2

PROTOCOL AMENDMENT(S):

1. On page 1 and 15, the Sponsor name and address should be as listed above. This amendment serves to clarify these sections of the protocol.
2. Page 15, Section B of Miscellaneous Information, the Lot No. for HI-MACS® should be "3854", per the Sponsor. This amendment serves to clarify this section of the protocol.
3. Page 15, under Report Handling, the report submission line should be "NA" and the GLP box should be checked. This amendment serves to clarify this section of the protocol.

Date Issued: 01/07/19 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 982-101

STUDY TITLE: Evaluation of Viral Elimination from a Solid Surface Material by Bleach or Alcohol – Human Immunodeficiency Virus Type 1 (HIV-1)	**STUDY DIRECTOR:** Tanya Kapes Tanya Kapes 1/7/19 Signature Date		
TEST MATERIAL(S): HI-MACS®	**LOT NO.** 3854	**DATE RECEIVED:** 11/29/18	**DS NO.** I681
DISINFECTANT(S): Diluted bleach 70% Isopropanol	 120718 NL#1220-18	 NA NA	 I681a I681b
PERFORMING DEPARTMENT(S): Virology and Toxicology	**STORAGE CONDITIONS:** Location: K2 ☐ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other:			
SPONSOR: LG Hausys Ltd. One IFC 10 Gookjegeumyoong-Ro, Yeongdeungpo-Gu Seoul, 150-876, Korea	**CONTACT PERSON:** Gene Lee E-mail: genelee@lghausys.com		

EXPLANATION(S):

4. The information for the disinfectants "diluted bleach" and "70% Isopropanol" were not provided on Project Sheet No. 1. They should be as written above. This amendment serves to clarify Project Sheet No. 1.