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Microbac Protocol

EVALUATION OF VIRUS ELIMINATION BY DISINFECTING PRODUCTS USING SURFACE MATERIALS FROM A BLOOD ANALYZER DEVICE

Duck Hepatitis B virus
(Surrogate for Human Hepatitis B virus)

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

Prepared for:
VoCare, Inc.
8888 Keystone Crossing
Suite 1300
Indianapolis, IN 46240

November 27, 2018

Microbac Protocol: VOC.1.11.27.18

Microbac Project: _____

OBJECTIVE:

This study is designed to evaluate virus elimination effectiveness of disinfecting products against duck hepatitis B virus (surrogate for human hepatitis B virus) on surface materials representative of a blood glucose monitoring system (BGMS) or Blood Analyzer Device. The test follows the ASTM E1053-11, "*Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces*", and ASTM E2362-15, "*Standard Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection*" with customization for towelette and viral testing. This study will be performed in compliance with the US Food and Drug Administration's Good Laboratory Practices (GLP) regulations (21 CFR 58).

TESTING CONDITIONS:

One disinfectant wipe product, one lot, will be evaluated for effectiveness in eliminating virus that is challenged and dried onto surface materials that represent the materials used to manufacture a BGMS (e.g., a lancing device) or other blood analyzer device. Twelve types of surface materials will be tested, each at three lots and 1 piece per lot.

The disinfectant product will be tested in a manner consistent with the disinfectant label directions. The liquid virus inoculum will be added to the surface of the material and allowed to dry. Then each test disinfectant wipe will be used to wipe down the surface material and held for a defined exposure (contact) time. One contact time will be tested for the disinfectant product. After a timed exposure period as indicated on the label of the disinfectant, the disinfectant-virus mixture on the surface will be neutralized, scraped off from the surface, collected, and assayed for the amount of surviving infectious virus using quadruplicate inoculation per dilution.

Note: per regulatory agency, it is unnecessary to test 3 lots of the disinfectant if it is an EPA-registered product and if the manufacturer instruction (contact times etc.) is followed. In this study, the disinfectant to be tested is an EPA-registered product with a Hepatitis B Virus claim; and the disinfectant manufacturer use-instructions will be followed, thereby only a single lot per disinfectant will be tested.

MATERIALS:

A. Test substances and surface carriers will be supplied by the sponsor of the study.

The disinfectant test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance must be specified by the sponsor prior to initiation of testing.

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

Microbac will retain all unused test substances and surface carriers for a period of one year upon completion of the test, then return them to the sponsor of the study or discard them following Sponsor approval 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): Duck Hepatitis B Virus (DHBV) (surrogate for human HBV). Strain: Grimaud; Source: HepadnaVirus Testing, Inc.

2. Host: Primary duck hepatocytes. Source: Metzer Farms

3. Laboratory equipment and supplies.

4. Media and reagents:

   Media and reagents appropriate to the virus-host system and appropriate neutralizer will be documented in the data pack and project sheets.

C. Material supplied by the Sponsor (see “Miscellaneous” page for details):

- Surface material coupons (Note: Appropriate parts directly from the test device may be used)

- Disinfectant wipes

TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be labeled with the following information: virus 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. Note: The product evaluation criteria are described later in this protocol.

A. Inoculum preparation:

Viral stocks are purchased from reputable sources. They are titered and stored in an ultra-low temperature (-60°C to -90°C) freezer. Records are maintained that demonstrate the origin of the virus.

Frozen viral stocks will be thawed on the day of the test. The serum content of the virus stock is 100% duck serum. No additional serum (such as fetal bovine serum) will be added to the virus stock.

B. Carrier (coupon) preparation:

Twelve types of surface materials will be tested for virus elimination using one disinfectant product. Coupons or parts representing 3 lots of each type of surface will be tested, at one piece or one group per lot.

When uniform-sized surface coupons are used, square coupons at 1 inch (2.54 cm) x 1 inch (2.54 cm) shall be used when possible (note: virus will be inoculated onto a 0.5 inch x 0.5 inch area as described below).

When parts of the BGMS or other blood analyzer device are used, the same type of parts must be used for both the test substance-treated and virus recovery control runs. For test surfaces that are smaller than 0.5 inch x 0.5 inch, virus will be inoculated onto the entire surface of outer side of the piece, up to an area of approximately 0.25 square inches.

For materials that are too small to be inoculated with 0.05 mL virus per piece, multiple pieces may be combined as one group and treated together.

Pre-test Surface Material Treatment:

All coupons will be Ultra-violet (UV) irradiated under a biosafety cabinet for a minimum of 15 minutes per side to reduce the bioburden.

Virus Challenge onto the Coupons:

The exact volume of challenge should be appropriate to the size of the coupon and must remain consistent between the test agent-treated samples and the virus recovery controls for all test materials.

For each piece of surface material, an aliquot of 0.05 mL stock virus will be applied onto each carrier using a micro-pipettor; and the virus is allowed to dry at ambient temperature. Other volume of virus inoculum may be used if the surface material is smaller than 0.5 inch x 0.5 inch. The virus drying time and temperature will be recorded. Each individual piece will be treated using a piece of towelette.

Only the side of the coupon that represents the outer surface of the device that may come into contact with blood will be contaminated with virus and treated with the disinfectant agent. Care will be taken to avoid over-flow of virus to outside the area to be tested. The entire virus-contaminated area will be sufficiently wiped following the procedure described in the below sections.

For materials that are too small to be inoculated with 0.05 mL virus for each single piece, multiple pieces will be tested as a group, where each piece will be inoculated with an aliquot of stock virus for a total of 0.05 mL virus per group by a micro-pipettor and allowed to dry at room temperature. The virus drying time and temperature will be recorded.

Number of Coupons to Be Tested:

Three carriers, or three groups of multiple pieces per group for small materials (one per lot at three lots) per each type of surface material will be prepared for each of the disinfectants. Three carriers, or three groups of multiple pieces per group for small materials (one per lot at three lots) per each type of surface material will be prepared for the virus recovery control. In addition, one carrier, or one group of multiple pieces per group for small materials (from a randomly chosen lot) will be prepared for each type of test surface for the cytotoxicity and

neutralizer effectiveness/viral interference controls using media challenge in lieu of virus.

C. Disinfectant agent preparation:

One disinfectant product (towelette) will be tested. The disinfectant does not require additional preparation as they are pre-saturated towelettes and ready-to-use.

D. Test:

One disinfectant product (towelette), one lot, will be tested on twelve types of surface materials, at three lots and one unit per lot for each material.

The disinfectant agent will be used to treat the virus-contaminated surface coupons as described below. Each coupon (or each group of multiple pieces, for small materials) will be treated by a separate piece of towelette.

Disinfectant towelettes handling:

For a disinfectant towelette test substance that is packaged in a canister, at the beginning of the testing, the canister will be inverted for at least 2-3 minutes to ensure wetness of the towelettes. Additionally, each canister should be inverted for 2-3 minutes approximately every 30 minutes throughout the testing to ensure consistent and thorough wetness of the towelettes.

When using towelettes from a new canister, the lid will be removed and the center of the roll will be pulled out and inserted into the lid opening. The lid will then be replaced and the towelettes pulled through the lid opening, making them ready for use.

The disinfectant test substance will be used to treat the virus-contaminated surface coupons as described below. Temperature will be monitored and recorded during the test.

Disinfection treatment:

The disinfectant wipe instruction may stipulate a pre-cleaning step for bloodborne viruses when heavy soil (such as blood) is present on surface. Although the DHBV inoculum is in 100% duck serum and considered heavy soil load, a pre-cleaning step is not recommended for viral inactivation studies by the regulatory agency as a "worst-case" scenario. Therefore, a pre-cleaning step will not be performed, unless otherwise directed by the Sponsor (see "Miscellaneous Information" page).

For the disinfection treatment process, each virus-contaminated surface coupon will be wiped with a piece of the disinfectant towelette with moderate pressure as follows. Only the side or areas of the coupon that has been contaminated with virus will be treated.

Dispense a fresh piece of towelette. Fold the towelette lengthwise twice and two to five times (depending on the size of the towelette and the surface coupon) inward beginning from the far end. Then the outside edges of the towelette will be pulled upward to form a "U" shape and grasped on one side with the thumb and the other side with the index and middle finger. Each contaminated test carrier will be wiped with the towelette with moderate pressure for 3 passes horizontally, then a new, unused area of the same piece of the towelette will be exposed and the coupon will be wiped again for 3 passes vertically. One pass is defined as a back and forth motion (for example, left to right and back to the left). Care will be taken to cover the entire virus-contaminated area. One towelette is used to clean one test carrier.

A stopwatch will be started immediately after the wiping is finished; and the coupon will be allowed to sit through the exposure (contact) time. Note: After the wiping application, the towelette will not be left sitting on the coupon during the contact time; but the test surface will be covered with a sterile lid to avoid excessive evaporation under the biosafety cabinet.

The coupon will be visually monitored for wetness throughout the contact time. To test as a worst-case scenario, no re-wiping will be conducted throughout the contact time, unless otherwise directed by the Sponsor (see "Miscellaneous Information" page).

A new pair of single-use gloves will be worn after each towelette treatment. The used towelette will be discarded.

Virus elution from carrier:

Upon completion of the contact time, the residual virus-disinfectant on the entire group of the carrier materials will be neutralized with a total of 1.0 mL of the neutralizer solution and the mixture will be scraped off from the surface with a cell scraper or another appropriate method.

The liquid neutralizer should cover all areas of the material that has been contaminated with virus.

This post-neutralized sample (PNS), considered Undiluted, will be serially diluted using dilution medium (DM). The sample will then be inoculated as described in Section E.

E. Infectivity assay:

The residual infectious virus in both test and controls will be detected by immunofluorescent staining targeting the S envelop protein of DHBV (DHBsAg).

Selected dilutions of the neutralized inoculum/test substance mixtures will be inoculated onto Primary duck hepatocytes (four wells per dilution per reaction mixture) and incubated at 36±2°C in 5±3% CO_2 overnight (approximately 20-30 hours) for viral adsorption. After adsorption, the monolayer will be refed with media and returned to the above listed incubation conditions for a total of 10-14 days. During the incubation phase the media may be replaced with fresh media every 2-4 days to maintain the cells. After incubation, the infectious DHBV will be assayed by immunofluorescence assay according to Microbac SOP M1006.VI.013 (current version).

F. Controls:

All controls will be performed at the same time as the test, incubated under the same conditions and assayed in the same manner as the test.

1. Virus recovery control (VRC)

   Three carriers (one piece or one group per lot at three lots) per each type of surface material will be performed for this control.

   The same amount of virus inoculum as used in the test agent runs will be applied onto the same number of piece(s) per type of material and let dry at ambient temperature. Then, the coupon will be held for the contact time without any treatment. Post the contact time, 1.0 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 (NE/VI):

   This control will be performed on each type of disinfectant test agent and each type of test surface (one lot, randomly selected), 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, 0.05 mL of media will be dried onto the coupon and then treated with the disinfectant test agent. Post the contact time and neutralization, the 1.0-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 (based on the projected cytotoxicity of the sample) 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 in Part D.

3. Cytotoxicity control (CT):

This control will be performed on each type of disinfectant test agent and each type of test surface (one lot, randomly selected), 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 media during the incubation phase of the study. This control will demonstrate that 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 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. Determination of the Limit of Detection (LOD):

The LOD will be determined by making a serial three-fold dilution of the virus stock, which will then be inoculated onto the host cells. Since the titer of the stock virus will be known from Section F.5, the LOD will be determined based on the highest dilution of the virus at which positive virus could still be detected.

H. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G. Arch. Exp. Pathol. Pharmakol. Vol. 162. Pages: 480-483, 1931) or other appropriate methods such as Reed and Muench, Am. J. of Hyg. 1938, 27:493. 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.

- Virus must be recovered from the neutralizer effectiveness/viral interference control (not exhibiting cytotoxicity).
- Cell viability control must exhibit absence of virus infectivity or test agent-induced cytotoxicity.
- The infectious virus units recovered from the Virus Recovery Control must be $\geq$ 4.0-Log_{10}.

PRODUCT EVALUATION CRITERIA:

According to the regulatory agencies, the test agent passes the test if there is complete inactivation of the virus at all dilutions. When non-viral cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling VA 20164.

REPORT FORMAT:

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

- Sponsor identification and test material identification
- Type of test 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 agent records, final report, and correspondence between Microbac and the Sponsor will be stored in the archives at Microbac Laboratories, Inc., 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 cell line monolayers used; media and reagent identified; 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 initiation date. All project sheets will be forwarded to the Sponsor.

Table 1 - Summary of samples to be assayed (1 of 4):

Sample No.	Test Agent	Test Surface	Sample Description
1	Disinfectant	Test Surface 1	Disinfectant, Test surface 1, lot 1
2			Disinfectant, Test surface 1, lot 2
3			Disinfectant, Test surface 1, lot 3
4		Test Surface 2	Disinfectant, Test surface 2, lot 1
5			Disinfectant, Test surface 2, lot 2
6			Disinfectant, Test surface 2, lot 3
7		Test Surface 3	Disinfectant, Test surface 3, lot 1
8			Disinfectant, Test surface 3, lot 2
9			Disinfectant, Test surface 3, lot 3
10		Test Surface 4	Disinfectant, Test surface 4, lot 1
11			Disinfectant, Test surface 4, lot 2
12			Disinfectant, Test surface 4, lot 3
13		Test Surface 5	Disinfectant, Test surface 5, lot 1
14			Disinfectant, Test surface 5, lot 2
15			Disinfectant, Test surface 5, lot 3
16		Test Surface 6	Disinfectant, Test surface 6, lot 1
17			Disinfectant, Test surface 6, lot 2
18			Disinfectant, Test surface 6, lot 3
19		Test Surface 7	Disinfectant, Test surface 7, lot 1
20			Disinfectant, Test surface 7, lot 2
21			Disinfectant, Test surface 7, lot 3
22		Test Surface 8	Disinfectant, Test surface 8, lot 1
23			Disinfectant, Test surface 8, lot 2
24			Disinfectant, Test surface 8, lot 3
25		Test Surface 9	Disinfectant, Test surface 9, lot 1
26			Disinfectant, Test surface 9, lot 2
27			Disinfectant, Test surface 9, lot 3

Table 1 - Summary of samples to be assayed (2 of 4):

Sample No.	Test Agent	Test Surface	Sample Description
28	Disinfectant	Test Surface 10	Disinfectant, Test surface 1, lot 1
29			Disinfectant, Test surface 1, lot 2
30			Disinfectant, Test surface 1, lot 3
31		Test Surface 11	Disinfectant, Test surface 2, lot 1
32			Disinfectant, Test surface 2, lot 2
33			Disinfectant, Test surface 2, lot 3
34		Test Surface 12	Disinfectant, Test surface 3, lot 1
35			Disinfectant, Test surface 3, lot 2
36			Disinfectant, Test surface 3, lot 3
37	None	Test Surface 1	VRC, Test surface 1, lot 1
38			VRC, Test surface 1, lot 2
39			VRC, Test surface 1, lot 3
40		Test Surface 2	VRC, Test surface 2, lot 1
41			VRC, Test surface 2, lot 2
42			VRC, Test surface 2, lot 3
43		Test Surface 3	VRC, Test surface 3, lot 1
44			VRC, Test surface 3, lot 2
45			VRC, Test surface 3, lot 3
46		Test Surface 4	VRC, Test surface 4, lot 1
47			VRC, Test surface 4, lot 2
48			VRC, Test surface 4, lot 3
49		Test Surface 5	VRC, Test surface 5, lot 1
50			VRC, Test surface 5, lot 2
51			VRC, Test surface 5, lot 3

VRC: Virus Recovery control

Table 1 - Summary of samples to be assayed (3 of 4):

Sample No.	Test Agent	Test Surface	Sample Description
52	None	Test Surface 6	VRC, Test surface 6, lot 1
53			VRC, Test surface 6, lot 2
54			VRC, Test surface 6, lot 3
55		Test Surface 7	VRC, Test surface 7, lot 1
56			VRC, Test surface 7, lot 2
57			VRC, Test surface 7, lot 3
58		Test Surface 8	VRC, Test surface 8, lot 1
59			VRC, Test surface 8, lot 2
60			VRC, Test surface 8, lot 3
61		Test Surface 9	VRC, Test surface 9, lot 1
62			VRC, Test surface 9, lot 2
63			VRC, Test surface 9, lot 3
64		Test Surface 10	VRC, Test surface 10, lot 1
65			VRC, Test surface 10, lot 2
66			VRC, Test surface 10, lot 3
67		Test Surface 11	VRC, Test surface 11, lot 1
68			VRC, Test surface 11, lot 2
69			VRC, Test surface 11, lot 3
70		Test Surface 12	VRC, Test surface 12, lot 1
71			VRC, Test surface 12, lot 2
72			VRC, Test surface 12, lot 3

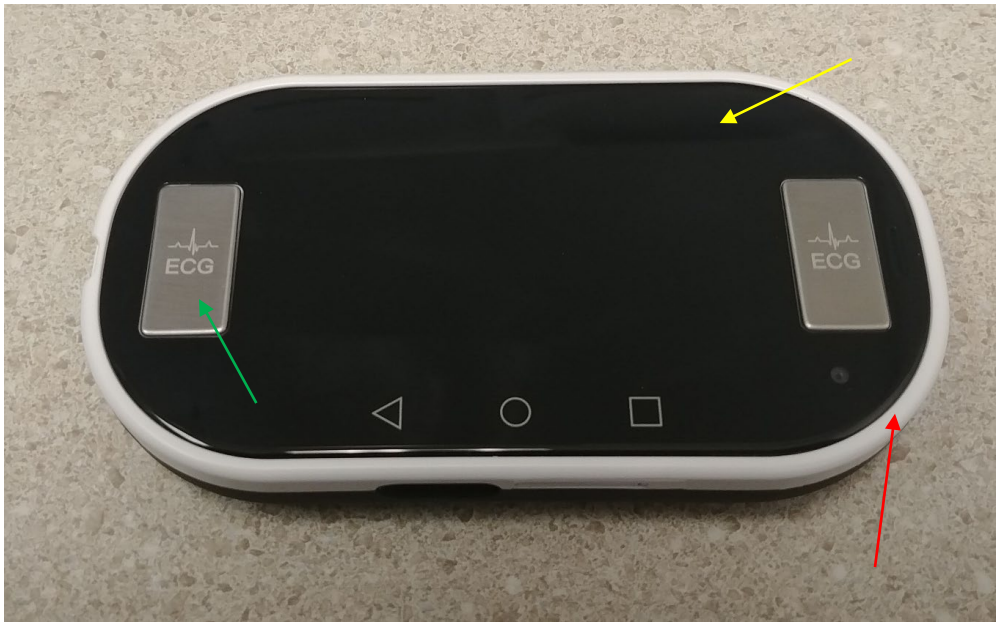
VRC: Virus Recovery control

Table 1 - Summary of samples to be assayed (4 of 4):

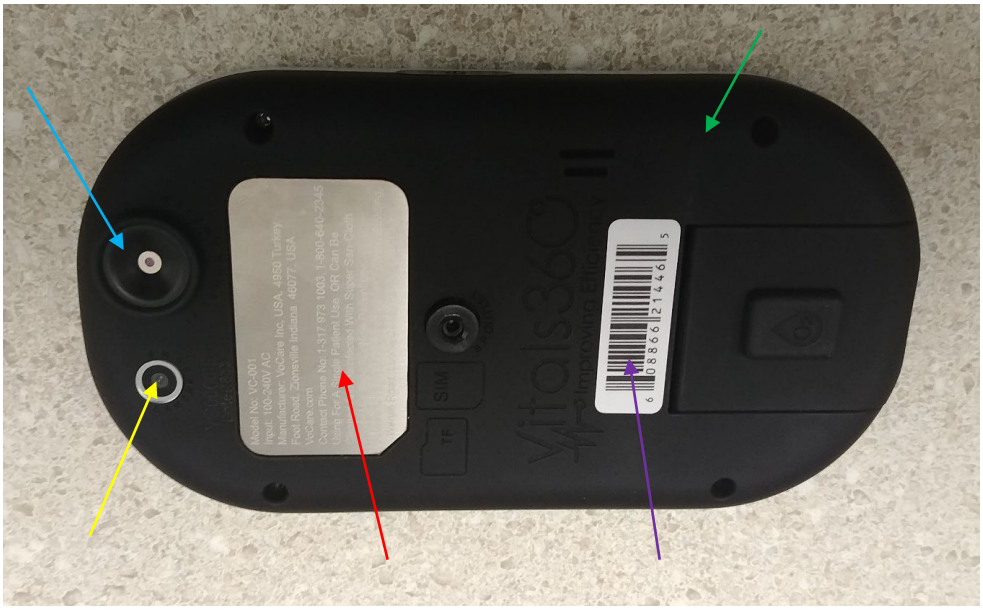
Sample No.	Test Agent	Test Surface	Sample Description
73	Disinfectant	Test surface 1	NE/VI control – Disinfectant, Test surface 1
74			TOX Control – Disinfectant, Test surface 1
75		Test surface 2	NE/VI control – Disinfectant, Test surface 2
76			TOX Control – Disinfectant, Test surface 2
77		Test surface 3	NE/VI control – Disinfectant, Test surface 3
78			TOX Control – Disinfectant, Test surface 3
79		Test surface 4	NE/VI control – Disinfectant, Test surface 4
80			TOX Control – Disinfectant, Test surface 4
81		Test surface 5	NE/VI control – Disinfectant, Test surface 5
82			TOX Control – Disinfectant, Test surface 5
83		Test surface 6	NE/VI control – Disinfectant, Test surface 6
84			TOX Control – Disinfectant, Test surface 6
85		Test surface 7	NE/VI control – Disinfectant, Test surface 7
86			TOX Control – Disinfectant, Test surface 7
87		Test surface 8	NE/VI control – Disinfectant, Test surface 8
88			TOX Control – Disinfectant, Test surface 8
89		Test surface 9	NE/VI control – Disinfectant, Test surface 9
90			TOX Control – Disinfectant, Test surface 9
91		Test surface 10	NE/VI control – Disinfectant, Test surface 10
92			TOX Control – Disinfectant, Test surface 10
93		Test surface 11	NE/VI control – Disinfectant, Test surface 11
94			TOX Control – Disinfectant, Test surface 11
95		Test surface 12	NE/VI control – Disinfectant, Test surface 12
96			TOX Control – Disinfectant, Test surface 12
97	NA	NA	Cell Viability Control
98	NA	NA	Stock Virus Titer Control

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

Test Surface Materials

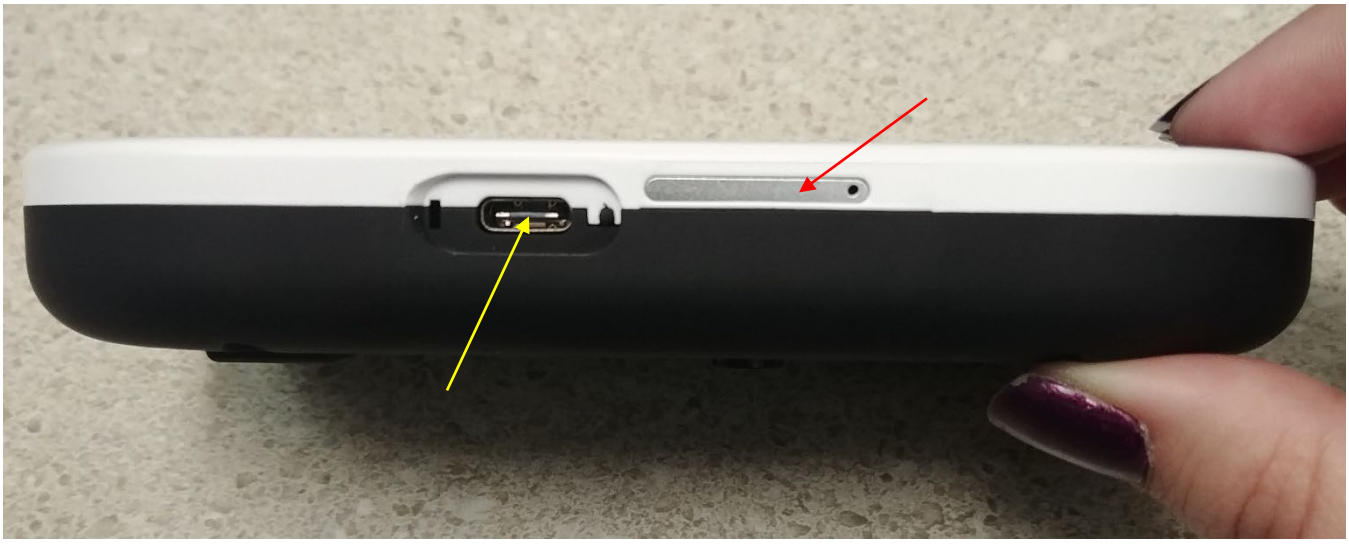


1) Glass screen
2) Metal pad
3) Plastic surrounding

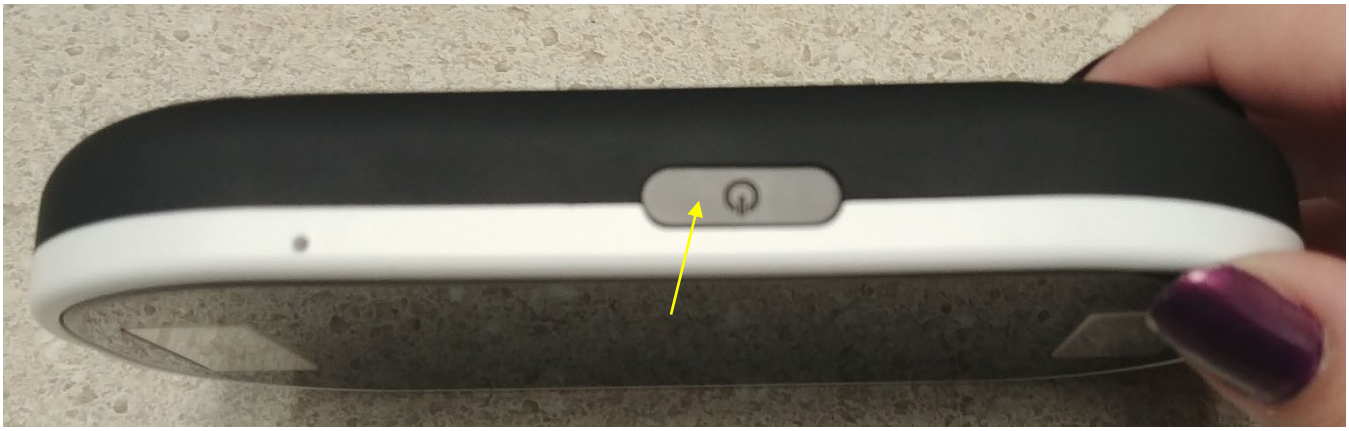


4) plastic rear casing
5) metallic label
6) matte label
7) lens
8) IR reader

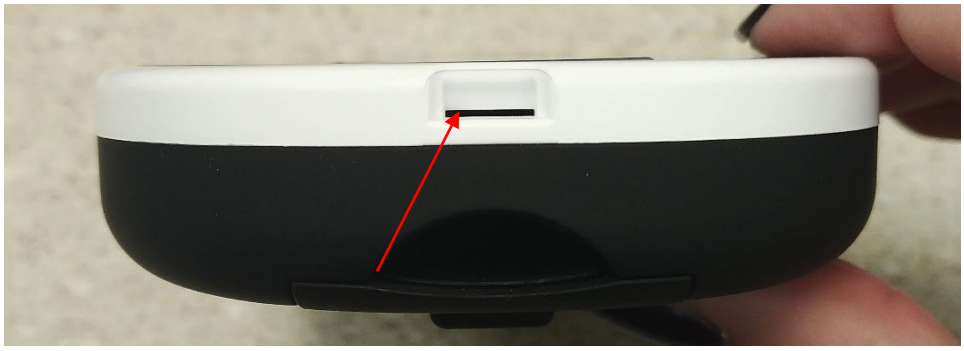
Test Surface Materials (continued)



9) USB port
10)SIM card cover



11)Power button



12) Strip port

MISCELLANEOUS INFORMATION:

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

A. Name and address: VoCare, Inc.
8888 Keystone Crossing
Suite 1300
Indianapolis, IN 46240

B. Disinfectant:

Choose one:	Disinfectant Product	Active Ingredients	Registr. #	Contact Time
☐	PDI Super Sani-Cloth® Germicidal Disposable Wipe	n-Alkyl (68% C12, 32% C14) dimethyl ethylbenzyl ammonium chlorides, 0.25%; n-Alkyl (60% C14, 30% C16, 5% C12, 5% C18) dimethyl benzyl ammonium chlorides, 0.25%); 55.0% Isopropyl Alcohol	9480-4	2 minutes
☐	Clorox Healthcare® Bleach Germicidal Wipes	0.55% Sodium Hypochlorite	67619-12	1 minute

Note: the batch (lot) number of the disinfectant will be recorded from product package.

Contact temperature: Ambient Temperature

C. Organic soil load: ≥ 5% serum (note: DHBV inoculum contains 100% duck serum)

D. MSDS of the disinfectant: To be available with the product package

E. The sponsor intends to submit this information to: FDA

F. Study Conduct: GLP

G. Test Surface Materials**:**

- See pages 17 & 18

Continued on next page

MISCELLANEOUS INFORMATION (Continued):

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature:_____ Date: _____

Printed Name: _____

STUDY DIRECTOR APPROVAL (Microbac):

Study Director Signature: _____ Date: _____

Printed Name: _____