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FINAL REPORT 2 of 3

VIRUCIDAL EFFICACY SUSPENSION TEST PER EN14476:2013+A2:2019 – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Test Product
Clinell Universal Wipes

Lot Number
MFB-020419-A

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

Sponsor
GAMA Healthcare Ltd
2 Regal Way
Watford, WD24 4YL
United Kingdom

Laboratory Project Identification Number
903-103

Clinell Universal Wipes 灭活新型冠状病毒检测报告

EN14476：2013+A2：2019– 新型冠状病毒

(SARS–CoV–2)(COVID–19 病)

检测样品

Clinell Universal Wipes

批号

MFB–020419–A

实验室

Microbac Laboratories, Inc.105 Carpenter DriveSterling, Virginia 20164

送检公司

英国伽玛卫生消毒用品有限公司

2 Regal Way Watford, WD244YL, UK

试验项目编号

903–103

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TEST SUMMARY

TITLE: VIRUCIDAL EFFICACY SUSPENSION TEST PER EN14476:2013+A2:2019 – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

STUDY DESIGN: This study was performed according to the signed Protocol and Project Sheet(s) issued by the Study Director. (See Appendix I)

OBJECTIVE: This test conforms in principle to the European Standard EN 14476:2013+A2:2019.

TEST MATERIALS: Clinell Universal Wipes, Lot No. MFB-020419-A, received at Microbac on 05/18/20 and assigned DS No. K660

SPONSOR: GAMA Healthcare Ltd
2 Regal Way
Watford, WD24 4YL
United Kingdom

测试摘要

标题： 病毒 功效 悬液 测试 每个人 EN14476：2013+A2：2019– 新型冠状病毒 (SARS–CoV–2)(COVID–19 病毒)

研究设计： 这项研究是根据研究主任签发的签署的议定书和项目表进行的。（见附录一）

目标： 本试验原则上符合欧洲标准 EN14476:2013+A2:2019。

试验材料： Clinell Universal Wipes，批号： MFB–020419–A，Microbac 实验室于 2020 年 5 月 18 日收到样品，并分配 DS 编号为 K660

送检公司： 英国伽玛卫生消毒用品有限公司
2 Regal Way Watford, WD244YL, UK

TEST CONDITIONS

Challenge virus:

Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), strain: USA-WA1/2020, Source: BEI Resources, NR-52281

Host:

Vero E6 cells, Source: ATCC CRL-1586

Active ingredient(s):

DDAC, BZK, 2-PE

Test condition storage condition:

Dark, at ambient room temperature

Test product appearance:

Wipe

Neutralizer(s):

D/E Broth (Pre-test Cytotoxicity Evaluation and Virucidal Quantitative Suspension Test)
Newborn Calf Serum (NCS) (Pre-test Cytotoxicity Evaluation)

Dilution medium:

Minimum Essential Medium (MEM) + 2% NCS

Contact time:

30 seconds; 60 seconds (Virucidal Quantitative Suspension Test)
60 seconds (Pre-test Cytotoxicity Evaluation)

试验条件

试验病毒株：

严重急性呼吸综合征相关冠状病毒 2(SARS–CoV–2)(COVID–19 病毒) 即新冠病毒，病毒株为 USA–WA1/2020，来源：BEI 资源，NR52281

宿主细胞：

Vero E6 细胞，来源：ATCC CRL–1586

有效成分：

二癸基二甲基氯化铵，苯扎氯铵，苯氧乙醇

试验条件贮存条件：

阴凉，在环境室温下测试

产品外观：

湿巾

中和剂：

试验前细胞毒性评价和病毒灭活定量悬浮试验 :

小牛血清 (NCS)（试验前细胞毒性评价）

稀释培养基：

MEM 培养基 +2%NCS

接触时间：

30 秒；60 秒（病毒定量悬液灭活试验)

60 秒 (试验前细胞毒性评价）

TEST CONDITIONS (continued)

Contact temperature(s):

20 ± 1°C (actual: 20°C) (Pre-test Cytotoxicity Evaluation and Virucidal Quantitative Suspension Test)

Interfering condition:

3 g/100 mL Bovine Serum Albumin (BSA) + 3 mL/100 mL erythrocytes (final concentration in reaction mixture: 3 g/L BSA + 3 mL/L erythrocytes) (“Dirty Condition”)

Dilutions tested:

Neat (i.e. ready-to-use)

Media and reagents:

MEM + 2% NCS
D/E Broth
NCS
MEM + 5% NCS
3 g/100 mL BSA + 3 mL/100 mL erythrocytes (10X Interfering Substance, “Dirty Condition”)

试验条件（续）

试验温度：

20±1° C(实际：20° C)（试验前细胞毒性评价和病毒定量悬液灭活试验）。

干扰条件：

3g/100mL 牛血清白蛋白 (BSA)+3mL/100mL 红细胞 (反应混合物中的最终浓度：3g/L BSA+3mL/L 红细胞)（“污染条件”）

稀释度测试：

整洁 (即用型产品)

培养基和试剂：

MEM+2%NCS

D/E Broth

NCS

MEM+5%NCS

3g/100mL 牛血清白蛋白 (BSA)+3mL/100mL 红细胞 (10 倍干扰物质，污染条件)

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164. The Pre-test Cytotoxicity Evaluation was lab initiated on 06/15/20 and concluded on 06/17/20. The Virucidal Suspension Test was lab initiated on 07/09/20 and concluded on 07/17/20. The study director signed the protocol on 06/15/20. 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 Microbac Laboratories, Inc. 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.

试验日期和机构

本试验的实验室阶段是在 Microbac 实验室进行， 实验室位于 105 Carpenter Drive, Sterling, VA 20164。 试验前细胞毒性评价于 2020 年 6 月 15 日开始，于 2020 年 6 月 17 日结束。 病毒灭活试验于 2020 年 7 月 9 日开始，2020 年 7 月 17 日结束。 研究主任于 2020 年 6 月 15 日签署了协议书。试验完成日期是研究主任签署最终报告的日期。

协议的所有更改或修订都有记录，由研究主任签署，保证协议的时效性。

要保存的记录

所有测试数据、协议、协议修改、测试材料记录、最终报告和 Microbac 实验室之间的通信、送检产品将存放在 Microbac 实验室， 实验室位于 105 Carpenter Drive, Sterling, VA 20164，或存放在现场以外的受控的机构。

EXPERIMENTAL DESIGN OVERVIEW

Inoculum preparation:

The stock virus was prepared by infection of Vero E6 cells. The cultures were frozen at -60 to -90°C several days after infection. After freezing and thawing, cell-free stocks were prepared by centrifugation. The stock virus was then aliquoted and stored at -60°C or below until used in testing. The virus stock was diluted 1.67-fold in MEM + 5% NCS prior to use in testing.

Test product preparation:

One concentration of the test substance was tested: “Neat” (ready-to-use).

Pre-test Cytotoxicity Evaluation:

In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of DM. 8.0 mL of test substance was added was added and mixed using a vortex. The reaction mixture was held for the contact time at 20°C. Following the contact time, a 1.0 mL aliquot of the reaction mixture was drawn up and neutralized in 1.0 mL of ice-cold neutralizer. This post-neutralized sample was further quenched to 1:10, 1:30, 1:100, 1:300, and 1:1000.

Virucidal quantitative suspension test:

In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of the virus suspension, carefully avoiding the upper sides of the dilution tube. Then, 8.0 mL of test substance was added and mixed using a vortex. The reaction mixture was then held for the contact time at 20°C. Following the contact time, a 1.0 mL aliquot of the reaction mixture was drawn up and neutralized in 1.0 mL of ice-cold neutralizer. This post-neutralized sample was further quenched 1:300 with ice-cold DM within 30 minutes.

Infectivity Assay:

Selected dilutions of the sample were inoculated onto the plates at 0.05 mL per well, 8 wells per dilution or 160 wells per dilution (for the Large Volume sample only) and incubated at 36 ± 2°C with 5 ± 3% CO_2.

实验设计概述

接种准备：

用 VeroE6 细胞感染制备了库存病毒。 培养物在感染后几天在 -60-90° C 处冷冻。冷冻解冻后，离心制备游离细胞库。 然后，库存病毒被均分并存储在 -60° C 或以下，直到用于测试。 病毒库存在 MEM+5%NCS 中稀释 1.67 倍，然后用于测试。

试验产品制备：

测试了一种浓度的测试物质：整洁 (即用型产品)

试验前测细胞毒性评价：

在一次试验操作中，10 倍干扰物质 1.0mL 与 1.0mL 的 DM 混合，加入 8.0mL 的试验物质，用振荡器混合。 反应混合物保持接触时间为 20° C。 在接触时间后，取 1.0mL 的反应混合物与 1.0mL 冰冷的中和剂混合进行中和。这个中和后的样品被进一步稀释到 1：10，1：30，1：100，1：300 和 1：1000。

病毒定量悬液试验：

在一次试验操作中，1.0mL 的 10 倍干扰物质与 1.0mL 病毒悬浮液的混合，小心地避免接触到稀释管的上部。 然后，加入 8.0mL 的试验物质，用振荡器混合。 然后将反应混合物保持在 20° C 的接触时间。 在接触时间后，取 1.0mL 的反应混合物与 1.0mL 冰冷的中和剂混合进行中和。 该中和后样品在 30 分钟内用冰凉 DM 进一步稀释到 1：300。

感染力鉴定：

将样品的选定稀释液接种到平板上，每个孔 0.05mL，每稀释 8 孔或每稀释 160 孔 (大容量样品)，并在 36±2° C 与 5%±3% 的 CO_2 孵育。

EXPERIMENTAL DESIGN OVERVIEW (continued)

The residual infectious virus in both test and controls was detected by viral-induced cytopathic effect (CPE). CPE is defined as cell rounding and sloughing off of the cell monolayer. After 8 days of incubation at 36 ± 2°C with 5 ± 3%, the plates were removed, read, and recorded for test-product specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

Virus Recovery Control (VRC):

This control was performed at time zero and at the end of the longer contact time. In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of the virus suspension, carefully avoiding the upper sides of the dilution tube. Then, 8.0 mL of DM was added and mixed using a vortex. 1.0 mL of the reaction mixture was immediately drawn up and neutralized in 1.0 mL of ice-cold neutralizer. The rest of the sample was held for the longer contact time at 20°C, then neutralized in the same fashion as above.

Neutralizer Effectiveness (NEC), Cytotoxicity (CT) and Viral Interference (VI) Controls:

This control was performed for each test substance using the longer contact time. In a single run, 1.0 mL of the 10X Interfering Substance was mixed with 1.0 mL of DM, carefully avoiding the upper sides of the dilution tube. Then, 8.0 mL of test substance was added and mixed using a vortex. The reaction mixture was then held for the longer contact time at 20°C. Following the contact time, a 1.0 mL aliquot of the reaction mixture was drawn up and neutralized in 1.0 mL of ice-cold neutralizer. This PNS was further quenched 1:300 with ice-cold DM within 30 minutes.

For Neutralizer Effectiveness: 4.5 mL of the post-quenched sample was spiked with 0.5 mL of stock virus and held in an ice-bath for 30 minutes. This was considered the 10^{-1} dilution. Selected dilutions were inoculated onto host cells as described in “Inoculation/Incubation”.

For Cytotoxicity: Selected dilutions were inoculated onto host cells as described in “Inoculation/Incubation”.

实验设计概述（续）

用病毒性细胞病变效应 (CPE) 检测试验和对照组的残留感染性病毒。病毒在细胞内增殖引起细胞变性、死亡裂解的作用称病毒的细胞病变效应。 在 36±2° C 和 5%±3% 的 CO_2 下孵育 8 天，取出、读取和记录测试产物特异性细胞毒性效应和 / 或病毒性细胞病变效应 (CPE)。

病毒恢复对照 (VRC)：

此对照试验较长接触时间结束时执行。 在一次试验操作中，1.0mL 的 10 倍干扰物质与 1.0mL 的病毒悬浮液混合，小心地避免接触到稀释管的上部。 然后，加入 8.0mLDM，用振荡器旋混合。 取 1.0mL 的反应混合物与 1.0mL 冰冷的中和剂混合进行中和。 其余样品在 20° C 处保持较长的接触时间，然后以与上述相同的方式中和。

中和剂有效性 (NEC)、细胞毒性 (CT) 和病毒干扰 (VI) 控制：

这种对照是用较长的接触时间对每种试验物质进行测试的。 在一次试验操作中，1.0mL 的 10 倍干扰物质与 1.0mL 的 DM 混合，小心地避免接触到稀释管的上部。 然后，加入 8.0mL 的试验物质，用振荡器混合。 然后将反应混合物在 20° C 保持较长的接触时间。 在接触时间后，取 1.0mL 的反应混合物与 1.0mL 冰冷的中和剂混合进行中和。 这种 PNS 在 30 分钟内用冰凉 DM 进一步稀释至 1：300。

中和剂的有效性: 4.5mL 的后淬火样品加入 0.5mL 的库存病毒，并在冰浴中保存 30 分钟。这被认为是 10–1 稀释。 选择的稀释液接种到宿主细胞上，即“接种 / 孵育”。

细胞毒性：选择稀释液接种到宿主细胞上，即“接种 / 孵育”。

EXPERIMENTAL DESIGN OVERVIEW (continued)

For Viral Interference: 0.05 mL of PBS and 0.05 mL of the lowest non-cytotoxic dilution of the sample was added to an appropriate number of aspirated host cell plates and pre-treated for 60 minutes at 36±2°C with 5±3% CO_2. Following this adsorption period, the sample was removed from the host-cell containing plate, and aliquots of 0.05 mL/well of the 10^{-3} – 10^{-8} dilutions of the stock challenge virus were added to the host-cell monolayer (8 wells per dilution).

Virus Stock Titer Control (VST):

The virus stock was used to make serial ten-fold dilutions in dilution medium.

Cell Viability Control (CVC):

This control was performed to demonstrate that the host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. 0.05 mL of DM was added to 8 wells of host cells and incubated in an identical manner as the test samples.

Large Volume (LV):

This sample was performed to increase the sensitivity of the assay. The PNS from the Virucidal quantitative suspension test for the “Neat” concentration was used to inoculate 160 wells of the host cell plates.

Inoculation/Incubation:

Each sample was used to make ten-fold serial dilutions in DM. 0.05 mL per well of appropriate dilutions were inoculated at eight wells per dilution or 160 wells per dilution (for Large Volume sample only) onto host-cell plates and incubated at 36±2°C with 5±3% CO_2 for 8 days.

实验设计概述（续）

对于病毒干扰：在适当数量的抽吸宿主细胞平板中加入 0.05mL 的 PBS 和 0.05mL 的最低非细胞毒性稀释的样品，在 36±2° C 与 5%±3% 的 CO_2 中预处理 60 分钟。 在此吸附期之后，将样品从含有宿主细胞的平板中去除，得到 0.05mL/ 孔、梯度稀释范围为 10^{-3} 至 10^{-8} 挑战病毒的稀释液，并添加到宿主细胞单层中（每个浓度稀释 8 孔）。

病毒库存效价对照 (VST)：

病毒库用于稀释培养基中连续稀释 10 倍。

细胞活力对照 (CVC)：

这种对照是为了证明宿主细胞仍然存活，并证实在整个潜伏期内使用的培养基的无菌性。在 8 孔宿主细胞中加入 0.05mL 的 DM，并以与试验样品相同的方式孵育

大容量 (LV)：

本样品用于提高测定的灵敏度。 用“Neat”浓度的病毒定量悬浮试验的 PNS 接种 160 孔宿主细胞平板。

接种 / 孵育：

每个样品被用来在 DM 中制造十倍的系列稀释液。 每孔 0.05mL 的浓度稀释液接种于 8 孔或 160 孔 (仅适用于大容量样品) 上，并在宿主细胞平板上孵育 8 天，36±2° C，5%±3% 的 CO_2。

CRITERIA FOR A VALID ASSAY

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied.

- Viral-induced cytopathic effect (CPE) was distinguishable from test product induced cytotoxic effect, if any.
- The difference of titer between the neutralized test product-treated and PBS-treated monolayers was ≤ 1.0 $\log_{10}$ in the viral interference control.
- The difference of titer between the NEC and VST was ≤ 0.5 $\log_{10}$.
- Virus was not detected in the cell viability control and was observed to be healthy and free from contamination at the end of the incubation period.
- The test virus suspension had a titer of at least 10^8 $TCID_{50}$/mL; or possessed at least a concentration which allowed the determination of a 4.0-$\log_{10}$ of the virus titer. The cytotoxicity of the product solution did not affect host cell viability in the dilutions of the test mixtures which was necessary to demonstrate a 4.0-$\log_{10}$ reduction of the virus.

PRODUCT EVALUATION CRITERIA

According to the EN14476:2013 + A2:2019 guidelines, the test product passes the test if there is at least a 4.0-log reduction in titer beyond the cytotoxicity level.

PROTOCOL AMENDMENTS / DEVIATIONS

PROTOCOL AMENDMENTS

See Appendix I.

PROTOCOL DEVIATIONS

No known procedural deviation from the study protocol or pertinent SOP’s occurred during the conduct of this study.

有效测定的标准

如果满足以下标准，测试将可用于评估测试结果。

- 病毒诱导的细胞病变效应 (CPE) 与试验产物诱导的细胞毒性效应（如果有的话）是可区分的。
- 在病毒干扰试验中，经中和处理的测试产品与经 pbs 处理的单层膜之间的滴度差≤ 1.0 log_{10}。
- NEC 与 VST 的滴度差≤ 0.5 log_{10}。
- 在细胞活力对照中未检测到病毒，并在潜伏期结束时观察到病毒是健康的，没有被感染。
- 试验病毒悬液滴度至少为 10^8 $TCID_{50}$ 或 至少具有允许测定的浓度至少有 4 个对数值的病毒滴度。 产品溶液的细胞毒性不影响宿主细胞的生存能力，在稀释的试验混合物中，这是必要的，以证明减少 4.0–log_{10} 的病毒。

产品评估标准

根据 EN14476：2013+A2：2019 指南，如果滴度超过细胞毒性水平，则测试产品通过测试。

协议修正 / 偏差协议修正

协议修正

见附录一。

协议偏差

在进行本研究期间，没有发生任何已知的程序偏离研究协议或相关 SOP 的情况。

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 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 ± 1.963 σ_m/2.

效价和 95% 置信区间的计算

$TCID_{50}$/mL 测定 , 使用以下公式的 Spearman- Karber 方法：

病毒库用于稀释培养基中连续稀释 10 倍。

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

注：

m = 有一半孔受感染的稀释倍数与测试体积的对数

x_k = 在所有培养物中诱导感染的最小剂量的对数

d = 稀释因子的对数

p_i = 稀释时阳性结果的比例 i

$\sum p_i$ =p 的和 i （从稀释倍数最高开始，产生 100% 的感染)

用 0.05 mL 的样品接种量转换为 $TCID_{50}$/ml。

报告每个样本的病毒滴度 ±95% 置信区间。 标准偏差 σ_m 使用以下公式计算：

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

.

注：

d_f = 稀释因子的对数

p_i = 稀释 i 时阳性结果的比例

σ_m = 标准偏差

n_i = 稀释时复制的数量 i

表示从第 k 次稀释开始的稀释量之和 , 95% 的置信区间为 m ± 1.963σ_m/2。

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

The below method (Poisson distribution) was used to perform the titer calculation:

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. 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.

效价和 95% 置信区间的计算（续）

采用以下方法（泊松分布）进行效价计算：

当一个样本含有低浓度的病毒时，有一个离散的概率，如果只对样本中的一小部分进行病毒测试，那么由于病毒在整个样本中的随机分布，该分数将测试为阴性。 分析的样本不含有传染性病毒的概率 p 用：p= (V–v)/V 。表示 y 其中 V 是容器的总体积，v 是被测试分数的体积，y 是随机分布在样本中的传染性病毒的绝对数量。 如果 V 相对于 v 足够大， 泊松分布可以近似 p：

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

其中 c 为感染性病毒浓度，v 为总样本量。 如果所有孔均为阴性，则认为该过程后的病毒滴度小于或等于此值。 样品测定的总体积为 v=v’nd，其中 v’是井中的测试体积，n 是每个样品的孔数，d 是样品稀释度。

RESULTS

Results are presented in Tables 1 – 6

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

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

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) x Volume Correction]

Table 1
Pre-Test Cytotoxicity Control – Newborn Calf Serum

Dilution*	Clinell Universal Wipes
	Neat
1:10	Cytotoxicity observed
1:30	Cytotoxicity observed
1:100	Cytotoxicity observed
1:300	No cytotoxicity observed
1:1000	No cytotoxicity observed

* Dilution refers to the fold of dilution from the PNS.

Table 2
Pre-Test Cytotoxicity Control – D/E Broth

Dilution*	Clinell Universal Wipes
	Neat
1:10	Cytotoxicity observed
1:30	Cytotoxicity observed
1:100	Cytotoxicity observed
1:300	No cytotoxicity observed
1:1000	No cytotoxicity observed

* Dilution refers to the fold of dilution from the PNS.

结果

结果见表 1-6

log_{10} 减少因子 (LRF) 的计算方法如下：

Log_{10} 降低因子 = 初始病毒载量 (Log_{10}) - 结束病毒载量 (Log_{10})

病毒载量的测定方法如下 :

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

表 1

预试验细胞毒性控制 - 小牛血清

稀释 *	Clinell Universal Wipes
	很整洁
1:10	观察到细胞毒性
1:30	观察到细胞毒性
1:100	观察到细胞毒性
1:300	未观察到细胞毒性
1:1000	未观察到细胞毒性

* 稀释是指国家统计局的稀释倍数。

表 2

预试验细胞毒性对照 -D/E 培养

稀释 *	Clinell Universal Wipes
	很整洁
1:10	观察到细胞毒性
1:30	观察到细胞毒性
1:100	观察到细胞毒性
1:300	未观察到细胞毒性
1:1000	未观察到细胞毒性

* 稀释是指国家统计局的稀释倍数。

RESULTS (continued)

Table 3
Titer Results – Test

Sample	Contact Time	Titer ($Log_{10}TCID_{50}$/mL)	Volume (mL)	Volume Correction[a]	Viral Load ($Log_{10}TCID_{50}$)
Virus Stock Titer Control	N/A	6.68 ± 0.20	-	-	-
Theoretical load (Virus Recovery Control) [b]	N/A	5.38 ± 0.20	10	2	6.68 ± 0.20
Theoretical load [b]	N/A	3.20 ± 0.20	10	300	6.68 ± 0.20
Cell Viability Control	no virus was detected; cells were viable; media was sterile				
Virus Recovery Control (VRC)	T = 0	5.80 ± 0.17	10	2	7.10 ± 0.17
	T = end	5.43 ± 0.18	10	2	6.73 ± 0.18
Neutralizer Effectiveness Control (NEC) [c]	60 seconds	6.43 ± 0.18	N/A	N/A	6.43 ± 0.18
Clinell Universal Wipes	30 seconds	≤ -0.43 *	10	300	≤ 3.05 *
Clinell Universal Wipes	60 seconds	≤ -0.43 *	10	300	≤ 3.05 *

[a] Volume correction accounts for the neutralization and quench of the sample post contact time.

[b] Based on the Virus Stock Titer (6.68 Log_{10} $TCID_{50}$/mL) minus Log_{10} (Volume x Volume Correction)

[c] A 4.5 mL aliquot of post-quenched sample was spiked with 0.5 mL of stock virus, mixed via vortex, and held in an ice-bath for 30 minutes.

N/A = Not Applicable

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

Note 1: The difference in Viral Load between the VST and NEC was ≤ 0.50 Log_{10} $TCID_{50}$.

Note 2: When no virus is detected in the "titration" sample, the "large volume" was used as the output load since large volume has a lower limit of detection (LOD). When virus was detected in the "titration" sample, the titration was used as the output load since titration was more accurate.

Table 4
Cytotoxicity Control

Sample	Dilution*	Cytotoxicity Control
Clinell Universal Wipes	10^0	No cytotoxicity observed

* The post-neutralized & quenched sample (PNS) was considered Undilute (10^0)

Conclusion: The neutralized test substance did not have significant cytotoxicity at undilute.

Table 5
Viral Interference Control

Sample	Virus Titer (Log_{10} $TCID_{50}$/mL)	Log_{10}Titer Difference
Clinell Universal Wipes	6.80 ± 0.23	0.13
PBS	6.93 ± 0.20	N/A

Conclusion: The neutralized test substance did not have significant viral interference.

表 3
滴度结果 – 测试

样本	接触时间	滴度 (Log_{10} $TCID_{50}$ /m L)	数量 (mL)	数量 更正 *	病毒负荷 (Log_{10} $TCID_{50}$ /m L)
病毒库存效价对照	n/a	6.68 ± 0.20	–	–	
理论负载（病毒恢复对照 ）[b]	n/a	5.38 ± 0.20	10	2	6.68 ± 0.20
理论负荷 [b]	n/a	3.20 ± 0.20	10	300	6.68 ± 0.20
细胞活力对照	未检测到病毒；细胞存活；培养基无菌				
病毒回收对照 (VRC)	T=0	5.80 ± 0.17	10	2	7.10 ± 0.17
	T= 结束	5.43 ± 0.18	10	2	6.73 ± 0.18
中和剂有效性对照 (NEC) [c]	60 秒	6.43 ± 0.18	n/a	n/a	6.43 ± 0.18
Clinell Universal Wipes	30 秒	⩽ –0.43 *	10	300	⩽ 3.05 *
Clinell Universal Wipes	60 秒	⩽ –0.43 *	10	300	⩽ 3.05 *

[a] 体积校正考虑了样品接触后时间的中和和淬火。

[b] 基于病毒原液滴度 (6.68 Log_{10} $TCID_{50}$/mL) 减去 Log_{10}(体积 x 体积修正)

[c] 4.5 mL 的淬火后的样品加入 0.5 mL 的储存病毒，通过振荡器混合，并在冰浴中保持 30 分钟。 N/A 即不适用

* 没有发现病毒；理论效价是根据泊松分布确定的。

注 1：VST 和 NEC 之间的病毒负荷差异为 0.50 ⩽对数 $_{10}$ $TCID_{50}$.

注 2：当“滴定”样品中没有检测到病毒时，使用“大容量”作为输出负载，因为大容量具有较低的检测极限 (LOD)。 当在“滴定”样品中检测到病毒时，滴定作为输出负载，因为滴定更准确。

表 4
细胞毒性对照

样本	稀释 *	细胞毒性控制
Clinell Universal Wipes	10^0	未观察到细胞毒性

* 中和后淬火样品 (PNS) 被认为是未稀释的 (100)

结论：中和试验物质对未蜕皮无明显细胞毒性

表 5
病毒干扰对照

样本	滴度 (Log_{10} $TCID_{50}$ /m L)	滴度差
Clinell Universal Wipes	5.43 ± 0.18	0.13
PBS	6.43 ± 0.18	n/a

结论：中和试验物质对未蜕皮无明显细胞毒性

RESULTS (continued)

Table 6
Viral Log_{10} Reduction

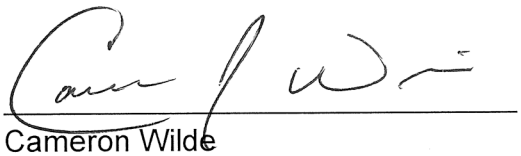
Test	Contact Time	Input Load ($Log_{10}TCID_{50}$)	Output Load ($Log_{10}TCID_{50}$)	Reduction ($Log_{10}TCID_{50}$)
Clinell Universal Wipes	30 seconds	7.10 ± 0.17	≤ 3.05	≥ 4.05 ± 0.17
Clinell Universal Wipes	60 seconds	7.10 ± 0.17	≤ 3.05	≥ 4.05 ± 0.17

CONCLUSIONS

According to the EN14476:2013+A2:2019 guideline, the test product passes the Virucidal Efficacy Suspension Test if there is at least a 4.0-log reduction in viral titer beyond the cytotoxicity level.

When tested as described, Clinell Universal Wipes, met the European Standard EN14476:2013+A2:2019 guideline, when Severe Acute Respiratory Syndrome-related Coronavirus was exposed to the test product for 30 seconds and 60 seconds at 20°C, with a Log_{10} reduction of ≥ 4.05p ± 0.17.

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

Study Director:  09/04/2020

Cameron Wilde Date

结果（续）

表 2

预试验细胞毒性对照 -D/E 培养

样本	接触时间	初始滴度 ($Log_{10}TCID_{50}$)	终末滴度 ($Log_{10}TCID_{50}$)	减少滴度 ($Log_{10}TCID_{50}$)
Clinell Universal Wipes	30 秒	7.10 ± 0.17	≤ 3.05	≥ 4.05 ± 0.17
Clinell Universal Wipes	60 秒	7.10 ± 0.17	≤ 3.05	≥ 4.05 ± 0.17

结论

根据 EN 14476:2013+A2 2019 标准，如果病毒滴度降低大于 4 个 log，超过细胞毒性水平，则所测样品通过灭活病毒定量悬液法测试。如所述，当新冠病毒（SARS-CoV-2）暴露于被测样品（Clinell Universal Wipes）30 秒和 60 秒 (20±1℃) 后，新冠病毒滴度减少 log_{10} ≥ 4.05±0.17, 符合 EN14476 灭活病毒的标准。

说明：以英文检测报告为准，中文译文仅供参考