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FINAL REPORT

VIRAL PENETRATION (STRIKE THROUGH) TEST

PROTOCOL NO. 200007603-01

LABORATORY NO. 163318

PREPARED FOR:

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VIRAL PENETRATION (STRIKE THROUGH) STUDY

LABORATORY NUMBER:	163318
PROTOCOL NUMBER:	200007603-01
SAMPLE SOURCE:	Audiology Products
SAMPLE IDENTIFICATION:	Sample #1: 0.35 to 0.50 mil LLDPE Sample #2: 1 mil LDPE high slip
DEVIATIONS:	Yes; refer to text
DATA ARCHIVE LOCATION:	Sequentially by lab number
TEST REQUESTED:	Viral Penetration (Strike Through)
PROTOCOL APPROVAL DATE:	04 May 2000
SAMPLE RECEIVED DATE:	08 May 2000
LAB PHASE START DATE:	11 May 2000
LAB PHASE COMPLETION DATE:	12 May 2000
REPORT ISSUE DATE:	18 May 2000
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REFERENCES:

Laufman, H., et.al. 1975. Strike-Through of Moist Contamination by Woven and Nonwoven Surgical Materials. Ann. Surg., June, P.875.

Schwartz, J.T. and Saunders, P.E. 1980. Microbial Penetration of Surgical Gown Materials. Surg. Gynecol. Obstet. 150:507.

INTRODUCTION:

This report describes the procedure followed for viral penetration testing of protective materials. The study design consisted of applying a viral suspension to the surface of the test materials. Each test material was placed onto the surface of agar assay plates overlayed with *Escherichia coli*. The challenge organism used in this study was ϕX174 bacteriophage (ATCC #13706-B1). The challenge level was approximately 10^6 plaque forming units (PFU) per test specimen. Test specimens were exposed to a 100 μL aliquot of the viral challenge for 8 hours at room temperature. Viral penetration was scored as the presence of plaques on the surface of the assay plates after incubation.

CHALLENGE PREPARATION - ϕX174:

Approximately 100 mL of nutrient broth (NB) was inoculated with *E. coli* and incubated 6-18 hours at 37 ± 2°C with rapid shaking. A 1:100 dilution of the culture was prepared and incubated at 37 ± 2°C. The culture was allowed to grow to a density of 2-4 x 10^8 CFU/mL. This cell density corresponded to an optical density of 0.3 - 0.5 on a spectrophotometer at 640 nm.

The bacterial culture was inoculated with 1 mL of the ϕX174 bacteriophage stock (ATCC #13706-B1) per 100 mL of culture. The suspension was incubated with rapid shaking for approximately 5 hours at 37 ± 2°C. Complete lysis of the host bacteria was noted when the broth cleared.

The virus suspension was centrifuged at 10,000 x *g* for 40 minutes. The supernatant was filtered through a sterile 0.2 μm filter to remove the host cell debris. The ϕX174 culture was refrigerated at 2-8°C.

BACTERIOPHAGE PLAQUE ASSAY:

The top agar was melted and maintained at 45 ± 2°C. Three mL of top agar and 1-2 drops of an overnight broth culture of *E. coli* C were added to sterile test tubes. Ten-fold serial dilutions of the bacteriophage were made in peptone water blanks and an aliquot of each sample was added to the top agar tubes. Three replicates per dilution or sample were tested. Each tube was vortexed and poured over the surface of a bottom agar plate. The plates were allowed to solidify, and then incubate at 37 ± 2°C for 18-24 hours. After incubation, the plaques were counted and the phage titer or sample results calculated.

TEST PROCEDURE:

All samples were sterilized prior to receipt. Sample type: .35 - .50 mil LLDPE was aseptically cut into approximately 80 mm diameter swatches from the sterile test material. Sample type: 1 mil LDPE high slip was received pre-cut into 80 mm disks.

Using sterile forceps and working in a HEPA hood, test specimens were placed onto the surface of prepared agar plates. Agar plates were prepared by mixing 3 mL of TOPAG with 1 to 2 drops of *E.coli* and pouring the mixture onto BOTAG plates. Plates were refrigerated until needed. Using a calibrated micro pipettor, 100 μL of challenge was placed onto the surface of each test swatch within 10-15 minutes of placement of the sample onto the plates. Assay plates were covered and allowed to sit at room temperature for approximately 8 hours. Three replicates were evaluated per sample type. After the test interval, the swatches were carefully removed and the plates incubated at 37 ± 2°C for 18-24 hours. Assay plates were scored for viral penetration. Plates with no plaques were scored as negative and plates with plaques were scored as positive.

CONTROLS:

A sample control, negative control, positive control, and growth promotion were included in the test system. A sample control (test material minus the challenge) was prepared for each material type. The sample control was included in the study to monitor the sterility of the test material and the aseptic technique of the technician.

A negative control (2 mil polyethylene film that has consistently not allowed ϕX174 penetration) was used to show that a negative result could be obtained when challenged with the ϕX174 bacteriophage.

A positive control was also included in the study to show that the ϕX174 bacteriophage could be recovered using the test procedure described. The positive control sample consisted of a 1-ply muslin substituted for the test material. The use of the 1-ply muslin is a deviation from the protocol which states a 2-ply muslin should be used. This change was made because 2-ply muslin does not allow microbial penetration.

A growth promotion control was prepared for each sample type. The growth promotion was used to ensure the media is capable of growing the challenge organism after exposure to the sample. This involved inoculating a sample control, minus the test material, with 0.1 mL of a ϕX174 bacteriophage challenge containing 10 to 100 CFU/0.1 mL.

DISCUSSION:

The material being tested will be used to manufacture keyboard covers. The ability of these covers to prevent viral penetration is of concern to potential users. This study integrated a viral challenge to the surface of the protective material to evaluate its ability to prevent viral penetration. This procedure employed one of the smallest known viruses (25-27 nm). The size of the ϕX174 bacteriophage provides a considerable margin of safety over major bloodborne pathogens like Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV). The ϕX174 bacteriophage is similar in size to the Hepatitis C Virus (HCV).

The viral suspension was diluted in a nutrient broth solution, prepared with surfactant-type Tween® 80, to provide a surface tension of approximately 42 ± 2 dynes/cm. Thereby, simulating the lower end surface tension range of blood and other body fluids.

RESULTS:

All three specimens of each material type demonstrated barrier capability for the full eight hours. The titer of the virus was 1.6×10^6 plaque forming units per device, which is within the acceptable range. All of the controls performed as expected.

Results are summarized in Tables 1 and 2.

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22 May 2000
Study Completion Date

KLP/rdb

TABLE 1. Viral Penetration (Strike Through) Results

SAMPLE ID: 0.35 - 0.50 mil LLDPE #1

SAMPLE IDENTIFICATION	GROWTH "+" NO GROWTH "0"
#1	0
#2	0
#3	0
Sample Control	0
Growth Promotion	+
Negative Control	0
Positive Control	+

TABLE 2. Viral Penetration (Strike Through) Results

SAMPLE ID: 0.35 - 0.50 mil LLDPE #1

SAMPLE IDENTIFICATION	GROWTH "+" NO GROWTH "0"
#1	0
#2	0
#3	0
Sample Control	0
Growth Promotion	+
Negative Control	0
Positive Control	+