# Needlefree Connector (NIP®) Microbial Challenge and Lipid Resistance

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## Microbial Challenge Study

IV Therapy is essential and common in today's medical treatment, but it is a well-known fact that it increases risks of device associated infections and accidental needle stick injuries. Needlefree alternatives can help reduce these risks.

Elcam Medical's Needlefree Connector (NIP®) is a stand-alone needlefree connector integrated with a swabbable Luer activated valve, allowing for maintenance of a fully closed connection through the entire use duration, as well as eliminating needle stick injuries.

Following is a summary of a study conducted to establish the microbial barrier properties of the device

## Study Purpose

To demonstrate that Elcam's Needlefree Connector provides full barrier protection against pathogens entering through the connector and the disinfection effectiveness of the swabbable valve with 70% alcohol.

## Method

The study was performed by Hy Laboratories Ltd., a certified microbiology laboratory in Rehovot, Israel.

The tested devices were stand-alone NIP® devices manufactured by Elcam that underwent pre-treatments of two EtO sterilization cycles and 50 days accelerated aging in 75$^0$C, equivalent to 5 years of natural aging according to Arrhenius model.

**The study included 5 groups:**
1 Test group (Inoculum + wiping) included 5 NIPs for each bacteria
3 Positive Control groups :
*Positive control I* (inoculated, not wiped) included 3 NIP units for each bacteria strain.
*Positive control II* (Immersion) included 1 NIP unit for each bacteria strain replaced daily.
*Positive control III* (Flow through) included 1 NIP unit for each bacteria strain replaced daily.
1 Negative control group (non- inoculated) included 5 units in total.

The study included **4 bacteria strains** -2 gram positive and 2 gram negatives – selected according to FDA guidance and the rationale in Table 1

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Table 1 **Challenge Microorganisms used in the Study**

| Rationale for Selection | Gram Stain | Challenge Microorganism |
|---|---|---|
| E.coli O157 ATCC 700728 | Negative, Rod | Aerobic bacteria, a human pathogen |
| Pseudomonas aeruginosa ATCC 9027 | Negative, Rod | Facultative anaerobic Bacteria.<br>A hospital acquired drug resistant pathogen |
| Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 43300 | Positive, Cocci | Facultative anaerobic Bacteria.<br>A hospital acquired drug resistant pathogen.<br>Major factor in catheter associated infections |
| Vancomycin-resistant Enterococcus faecalis (VRE) ATCC 51299 | Positive, Cocci | Facultative anaerobic Bacteria.<br>A hospital acquired drug resistant pathogen |

All the microorganisms used in the assay were prepared and stocked according to the Hy Laboratories specifications. Fresh stock of all 4 microorganisms was prepared for each inoculation day. Serial dilutions from the bacterial cultures were performed with saline solution to obtain $10^7$ cfu/ml per inoculum.

## Study Procedures

The study lasted 11 days and included 7 operation days (activation – inoculation- collection and Plating): Days 1-5, day 7 and day 11.

## Test group

**Activation (Simulated Use):** Each NIP was wiped with 70% alcohol for 15 sec, and was left to dry for at least 1 min. The valve was activated 4 times by attaching a sterile syringe and disconnecting it. The next step was performed after 1 hour. Activation was repeated three times (total activations=12 for each inoculation day, 84 total activations of each tested device for the entire study period).

**Inoculation:** Each NIP was wiped with 70% alcohol for 15 sec' and was left to dry for at least 1 min. 10µl from an estimated concentration of $10^7$ cfu/ml of each of the challenged bacteria was placed on the valve of the NIP (resulting in an inoculation of $10^5$ CFU/NIP), and left untouched for at least 1 min. Each NIP was wiped with 70% alcohol for 20 sec' and was left to dry for at least 1 min.

**Collection:** A 10ml saline with 0.1% polysorbate 80 (Tween 80) syringe was connected to the tested NIP, and the saline was pushed through the NIP into a sterile collection tube placed under the tested NIP. The collected saline was filtered through 0.45 µm membrane filter and the filter was placed on Trypticase Soy Agar (TSA) plate for bacterial growth.

**Incubation & quantification:** TSA plates were incubated for up to 3 days at 30-35 °C or until reliable counts were obtained. Following the incubation period, the number of colony forming units (CFU) that grew on each membrane filter was counted.

## Positive Control Groups

**Positive control I (Push through):** This Positive Control was performed as the Test, omit the alcohol disinfection step following inoculation. Following the saline collection, serial dilutions were performed to obtain dilution of $10^{-2}$ and $10^{-3}$. The dilutions were filtered as described in Test group.

**Positive control II (Immersion):** The aim of this control was to collect the bacteria placed on the NIP in case the drop slides away from the top of the NIP and will not be washed when saline is pushed through. Activation and inoculation were performed as in Positive Control I group. The tested device was transferred to a collection tube containing 20ml saline with 0.1% polysorbate 80 and vortexed for 1 min. Serial dilutions were performed and filtered as described above for Positive Control I.

**Positive control III (Flow through):** The aim of this control was to demonstrate that bacteria are not trapped within the NIP's body. Activations were performed as described in Test group. For each of the challenged bacteria, 10ml of saline with 0.1% polysorbate 80 was inoculated with 10µl from an estimated concentration of $10^{7}$ cfu/ml (for estimated total count of $10^{5}$ cfu). The inoculated saline was pushed through the NIP into a sterile collection tube placed under the tested NIP. Serial dilutions were prepared and filtered as described for Positive Control I.

The microbial count of all the Positive Controls groups was extrapolated to estimate the initial inoculums value using the dilution factor and expressed as CFU.

## Negative control

The negative control was performed as the test. Sterile saline was used for the inoculation step.

## Results

During the 11 days and after total 84 accesses and repeated activations of each tested device in the method described above, **Elcam's tested NIPs as well as the negative controls demonstrated no growth of the studied bacteria.**

Recovery of bacteria in control treatments was as expected and demonstrated recovery ranges as shown in Table 2

Table 2 **Bacterial recovery ranges in positive control groups**

| Bacteria | Recovery range (CFU) |
|---|---|
| E.coli | $2.84 \times 10^5 - 1.58 \times 10^6$ |
| P. aeruginosa | $4.8 \times 10^4 - 7.5 \times 10^5$ |
| MRSA | $2 \times 10^3 - 1.34 \times 10^6$ |
| VRE | $5.6 \times 10^4 - 2.18 \times 10^6$ |

## Conclusions

According to the study results we can conclude that Elcam's NIP connector provides full barrier protection against typical pathogens found in hospital environment when combined with proper external disinfection of the swabbable valve with 70% alcohol swab.

## Lipid Resistance Study

Lipid induced cracks and leakages are considered under-reported adverse events or mistakenly attributed to other circumstances. Needlefree connectors like Elcam's NIP® not only need to serve as a bacterial barrier and eliminate needle stick injuries, but also must maintain their integrity and functionality when exposed to lipids that are part of certain IV Therapy regimens.

Following is a summary of a study conducted by Elcam to establish the lipid resistance properties of the device.

## Study Purpose

To demonstrate that Elcam's Needlefree Connector (NIP®) is resistant to lipid substances for at least 7 days.

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## Method

The tested devices were stand-alone NIP® devices manufactured by Elcam that underwent pre-treatments of two EtO sterilization cycles and 50 days accelerated aging in 75$^0$C, equivalent to 5 years of natural aging.

- 60 devices were tested
- Both female and male Luers were tested in each device.
- Screwing torque was 40 In*OZ.
- For Female luers – 5 disconnections and 5 connections were preformed daily and the devices were examined every 24 hours from Day 1 until Day 12
- For male Luers - 1 disconnection and 1 connection were preformed daily and the devices were examined every 24 hours from Day 1 until Day 12

## Acceptance criteria

no leakage or cracks that can cause leakage after exposure to lipid for more than 48 hours up to 288 hours (12 days).

## Results

All tested devices withstood the acceptance criteria for 9 days. No leakage was found after exposure to lipid up to 216 hours (9 days).

## Conclusions

**According to the study results we conclude that Elcam's NIP® NeedleFree Connector is approved for use with lipids for 7 days.**

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