PYROGENIC RISK IN REUSING SUDs: EFFICIENCY OF PLASMA STERILIZATION IN REMOVING ENDOTOXINS FROM ELECTROPHYSIOLOGY CATHETERS

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• Pyrogens and endotoxins
• LAL test
• SUDs reprocessing – electrophysiology catheters
• Plasma sterilization
• Experimental study
  • Retrieval Assay
  • Sample testing
  • Inoculated sample testing
  • Depyrogenation testing
• Endotoxins are part of the outer membrane of the cell wall of Gram-negative bacteria. Endotoxins are invariably associated with Gram-negative bacteria whether the organisms are pathogens or not. The term "endotoxin" is properly reserved to refer to the lipopolysaccharide complex associated with the outer membrane of Gram-negative bacteria.

Structure of the cell surface of Gram-negative bacteria

Pyrogens and endotoxins

LAL test

SUDs reprocessing

Plasma sterilization

Experimental study

Retrieval assay

Sample testing

Inoculated sample testing

Depyrogenation testing
Pyrogens and endotoxins

- The injection of living or killed Gram-negative cells, or purified LPS, into experimental animals causes a wide spectrum of nonspecific patho-physiological reactions such as:
  - fever,
  - changes in white blood cell counts,
  - disseminated intravascular coagulation,
  - hypotension,
  - shock and death.

- European Pharmacopoeia requires that injectable substances and critical medical devices that come in contact with blood are non-pyrogenic.

- According to both US and European Pharmacopoeias, the limit for non-pyrogenic medical devices is fixed at 20EU per device [FDA, 1987]
Replaced the former “rabbit test”
  – Rabbits, like use, are sensitive to endotoxin and if a suspect sample of saline injected into a rabbit caused a fever then it is contaminated

Within these last 20 years, the use of *Limulus* amoebocyte lysate to detect and control the presence of pyrogenic substances in pharmaceuticals and medical devices has gained wide international acceptance and both the United States and European Pharmacopoeias contain descriptions and requirements for the LAL bacterial endotoxin test.
“Reprocessing as a practice is generally perceived to mean the cleaning, disinfection and sterilization of a medical device, including related procedures, as well as the functional testing and repackaging, carried out on a medical device after it has been put into service”

[EU Public Consultation, 2007]

Reprocessing is aimed at complete recovering of hygienic and functional requirements

If a manufacturer, a hospital, or a third party reprocessor labels a medical device as “non-pyrogenic”, this claim must be supported by conducting pyrogen testing by the USP pyrogen test. In November 1977, the FDA made the LAL test a valid in vitro assay for endotoxins available to medical devices for end-product (end-process) device testing [FDA, 1977]

The use of the LAL test could be helpful at monitoring the pyrogenic status of medical devices after reprocessing for safe reuse.
Electrophysiology catheters

- Non-lumened percutaneous catheters for electrophysiology and cardiac ablation
The regeneration process was based on three sequential treatments:

- **Decontamination** was provided following Italian legislation by 10 min immersion in a chlorine solution (sodium dichloroisocyanurate, 5000 ppm of free chlorine).

- Tap water rinsing

- Chemical **cleaning** by immersion in enzymatic detergent (10 min, 1% in water, at 40°C).

- Manual brushing and wiping of the exposed device surfaces by using non-linting tissue moistened in neutral soap water solution.

- Tap water rinsing,

- Drying by non-linting tissue

- Double packaging in Tyvek pouches

- **Sterilization** hydrogen peroxide gas-plasma (Sterrads 100S, Advanced Sterilization Products, Johnson and Johnson Inc., 54 min cycle).

[Tessarolo et al, 2006]
Plasma sterilization

- Sterrad technology is based on chemical sterilization by vaporized hydrogen peroxide (58%) and a detoxifying plasma cycle obtained by the application of radiofrequency.

[Moisan 2001, Jacobs 1993]
We monitored the device pyrogenic status in three fundamental steps of the reprocessing protocol:

- after clinical use,
- after decontamination-cleaning treatments,
- after complete reprocessing including sterilization by hydrogen peroxide gas plasma. (@ 20 EU)

A worst case scenario was simulated by reprocessing and testing catheters inoculated with high amount of endotoxins (40-300 CSE)

Moreover, depyrogenation tests were performed in order to evaluate the depyrogenation efficiency of the sole hydrogen peroxide sterilization treatment
Experimental study

• First: validate a suitable, efficient and reproducible method to elute endotoxins from device surface
• Second: apply this methodology to real samples.

61 cardiac electrophysiology and ablation non-lumen catheters produced by the major worldwide manufacturers were collected during 2 months of local cardiology department activity (S. Chiara Hospital, Trento, Italy) after clinical use.

Table 1: Cardiac electrophysiology catheters analysed in the study

<table>
<thead>
<tr>
<th>Device Type/s (manufacturer)</th>
<th>RA</th>
<th>PU</th>
<th>PC</th>
<th>PR</th>
<th>IS</th>
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<tbody>
<tr>
<td>Marin™ CS 7Fr (Medtronic Inc.)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
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<tr>
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<td>5</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>4</td>
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<tr>
<td>RF Conduct™ 7Fr (Medtronic Inc.)</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Stinger™ 8mm tip (Bard Inc.)</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>1</td>
<td>2</td>
<td>6</td>
<td>2</td>
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<tr>
<td>Celsius™ D/DS type (Biosense-Webster Inc.)</td>
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<tr>
<td>NaviStar™ F type 7Fr (Biosense-Webster Inc.)</td>
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<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Livewire TC™ (St. Jude Medical Inc.)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Total devices tested</td>
<td>15</td>
<td>7</td>
<td>10</td>
<td>15</td>
<td>9</td>
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</tbody>
</table>

RA, retrieval assay; PU, post use; PC, post cleaning; PR, post reprocessing; IS Inoculated samples.
A retrieval method, optimised to maximize endotoxin recovery efficiency, was assayed on 15 catheters spiked with a known amount of CSE units to obtain the average percent ratio of endotoxins recovery. This ratio was subsequently considered at defining proper dilutions for LAL test and actual quantities of EU in elution of real samples.

- Decontaminated and cleaned shafts were separated from catheter handler by using disinfected pliers, cut into 1–2 cm pieces, and placed in standard sterilization pouches for steam depyrogenation (180 min at 121°C).

- Depyrogenated samples were aseptically transferred to pyrogen-free screw-cap containers with 40 ml of a preheated 0.01% wt. SDS solution at 40°C. Endotoxin recovery with high temperature and surfactants is higher than sole water [Rioufol et al., 1999; Ross and Twohy, 1985].

- 40 CSE units were added as spiking quantity.
  - vortex 5 min
  - sonication 45 min at 40°C,
  - vortex for 1 min.
Retrieval Assay

- A set of two-fold serial dilutions was realized providing sensitivity to 5, 10, 20, 40, and 80 EU per sample.

- Inoculation of LAL vials and incubation at 37±1 °C, for 60±2 min.

- All dilutions and tests were performed in duplicate.

- The endpoint assay value for each set was the lowest endotoxin concentration at which the lysate formed a solid gel cloth.
Retrieval Assay: results

The log_{10} of each endpoint assay value was determined and values from the 30 sets were averaged to calculate the geometric mean endpoint for retrieval.

Average retrieval (EU/sample) 17.81

Average retrieval % (on 40 inoculated CSE) 44.5%

<table>
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<tr>
<th>Catheter type</th>
<th>Lot</th>
<th>1:1 (5 EU)</th>
<th>1:2 (10 EU)</th>
<th>1:4 (20 EU)</th>
<th>1:8 (40 EU)</th>
<th>1:16 (80 EU)</th>
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<tr>
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<tr>
<td>Stinger™ 8mm tip</td>
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<td>+</td>
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<td>+</td>
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<tr>
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<tr>
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<tr>
<td>Torq™ Josephson type</td>
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<tr>
<td>Torq™ Courmand type</td>
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<td>+</td>
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<tr>
<td>NaviStar™ F type 7Fr</td>
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<tr>
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<tr>
<td>Livewire TCS™</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Livewire TCS™</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Sample testing

- 32 whole catheter shafts
  - 7 untreated,
  - 10 decontaminated-cleaned,
  - 15 fully reprocessed

- Cut into 1-2 cm pieces and aseptically transferred to the same type of pyrogen-free screw-cap container used in the retrieval assay.

- Identical procedure for elution but no CSE added.

- Two dilution:
  - 1:1 11 EU/device (highest sensitivity)
  - 1:1.8 20 EU/device.

- In case of positive results, further twofold dilutions were tested until the end-point.

- Each analysis was performed in duplicate.
Sample testing: results

Pyrogenic status (positive if >20 EU/sample):

After Clinical Use positive 0% (0/7)
The analysis on the seven clinically used but untreated catheters showed no positive samples at both the dilution tested.

After cleaning and decontamination positive 10% (1/10)
Tests on decontaminated and cleaned catheters showed that two out of 10 samples were positive at 11EU per device and one out of 10 samples was positive at 20EU per device.

After reprocessing positive 0% (0/15)
LAL testing of reprocessed catheter did not give any test gelation (positive test) either to 20 and to 11EU per sample, indicating that all 15 reprocessed devices were non-pyrogenic according to the limit of 20EU per device.
All tests performed in duplicate were in accordance.
Pyrogenic status (positive if >20 EU/sample):

After cleaning and decontamination positive 10% (1/10)
Tests on decontaminated and cleaned catheters showed that two out of 10 samples were positive at 11EU per device and one out of 10 samples was positive at 20EU per device.
Inoculated sample testing

- In vitro inoculation of nine catheters
  - 3 catheters with 120 CSE units
  - 3 catheters with 600 CSE units
  - 3 catheters with 1000 CSE units

- CSE units were solvated in 95% ethanol, dropped on shafts and dried under vacuum at 60 Torr.

- Each catheter was cut in three equivalent pieces.

- One part was reprocessed and two remaining samples underwent no treatments.

- Treated and untreated samples were tested following the reported method of elution at 20 and 11EU per sample.
Inoculated sample testing: results

- LAL test performed on inoculated samples showed that the real amount of residual CSE on inoculated catheters at 40, 200, and 333 CSE units was respectively 40, 80, and 200 CSE units per device.

- Inoculated and reprocessed samples did not show any positive reaction of gelation either to 20 EU per sample and to 11 EU per sample in all of the three different quantities of inoculum, thus resulting as non-pyrogenic devices.
Depyrogenation testing

• Depyrogenation testing was performed according to USP requirement and CSE manufacturer’s recommendations.
• A glass vial containing 2500EU was opened, sealed in a double standard Tyvek sterilization pouch and sterilized in a Sterrads 100S hydrogen peroxide gas-plasma sterilizer.
• After sterilization, the CSE in the vial was reconstituted with 5ml of pyrogen-free water and a two-fold serial dilutions set was realized ranging from a sensitivity of 0.125–64EU per ml of the solution.
• All dilutions and tests were performed in duplicate. The endpoint assay value was considered and the respective endotoxins reduction ratio was computed.
Depyrogenation testing results

- The initial amount of 2500 CSE units was reduced to less than 40 units (8 CSE/ml in 5 ml of rinsing solution).
- Hydrogen peroxide sterilization by Sterrads 100S led to a CSE reduction higher than 62 times.

### Table 4: Results of depyrogenation test

<table>
<thead>
<tr>
<th>Dilutiona (sensitivity)</th>
<th>CSE units reduction (times)</th>
<th>LAL Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lot I</td>
</tr>
<tr>
<td>1:1 (0.125 EU/ml)</td>
<td>4000</td>
<td>+</td>
</tr>
<tr>
<td>1:2 (0.25 EU/ml)</td>
<td>2000</td>
<td>+</td>
</tr>
<tr>
<td>1:4 (0.5 EU/ml)</td>
<td>1000</td>
<td>+</td>
</tr>
<tr>
<td>1:8 (1 EU/ml)</td>
<td>500</td>
<td>+</td>
</tr>
<tr>
<td>1:16 (2 EU/ml)</td>
<td>250</td>
<td>+</td>
</tr>
<tr>
<td>1:32 (4 EU/ml)</td>
<td>125</td>
<td>+</td>
</tr>
<tr>
<td>1:64 (8 EU/ml)</td>
<td>62.5</td>
<td>-</td>
</tr>
<tr>
<td>1:128 (16 EU/ml)</td>
<td>31.2</td>
<td>-</td>
</tr>
<tr>
<td>1:252 (32 EU/ml)</td>
<td>15.7</td>
<td>-</td>
</tr>
<tr>
<td>1:512 (64 EU/ml)</td>
<td>7.6</td>
<td>-</td>
</tr>
</tbody>
</table>
Summary and conclusions

- Standard clinical use did not represent a source for endotoxin contamination,
- Tap water and manual cleaning processing could increase the pyrogenic load in a significant way.
- Sterilization by hydrogen peroxide gas plasma resulted in effective reduction of the endotoxin contamination and in safe reprocessing of 15/15 clinically used catheters.
- Pyrogenic loads of 40, 80, 200 EU/device were reduced to less than 11EU/device.
- Depyrogenation testing showed efficiency in endotoxin reduction of more than 62 times (1.8 log).

Hydrogen peroxide gas-plasma sterilization could be considered for reprocessing electrophysiology catheters obtaining non-pyrogenic reprocessed devices.
Acknowledgments

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  - CSSD S. Chiara Hospital in Trento

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Thank you for your attention!