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# Evaluation of the endotoxin risk posed by use of contaminated water during sterilisation of surgical instruments

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# The issues are the following :

- Is there any risk for the patient, associated with contaminated water, at a point or the other of the sterilisation process ?
- Can we determine any threshold concerning the number of micro-organisms that can be accepted in the different waters used, without causing any risk for the patient ?
- If the micro-organisms are eliminated along the different steps of the sterilisation process, including cleaning, is there any risk associated with endotoxins ?

→ *no data were currently available*

# Different points to be checked

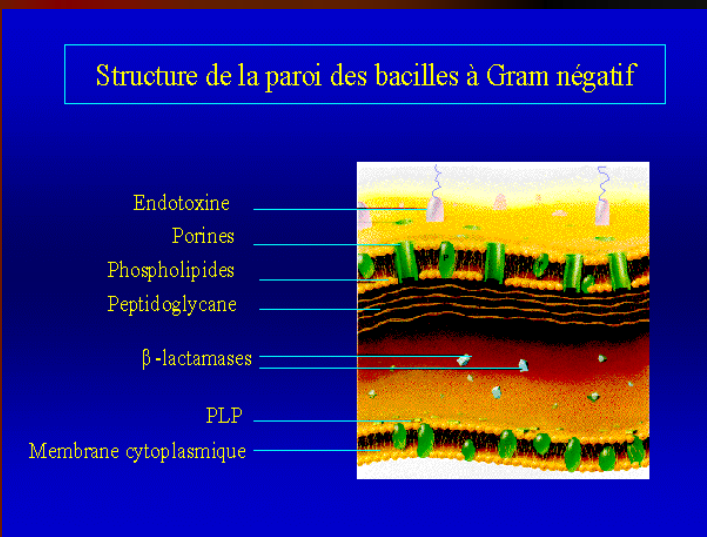
- Endotoxins : transmission and monitoring : injectable fluids, catheters, dialysis

- Still unknown :
  - After use, are surgical instruments contaminated ?
  - Can water or eventually steam contaminated by endotoxins transmit them to the patient ?

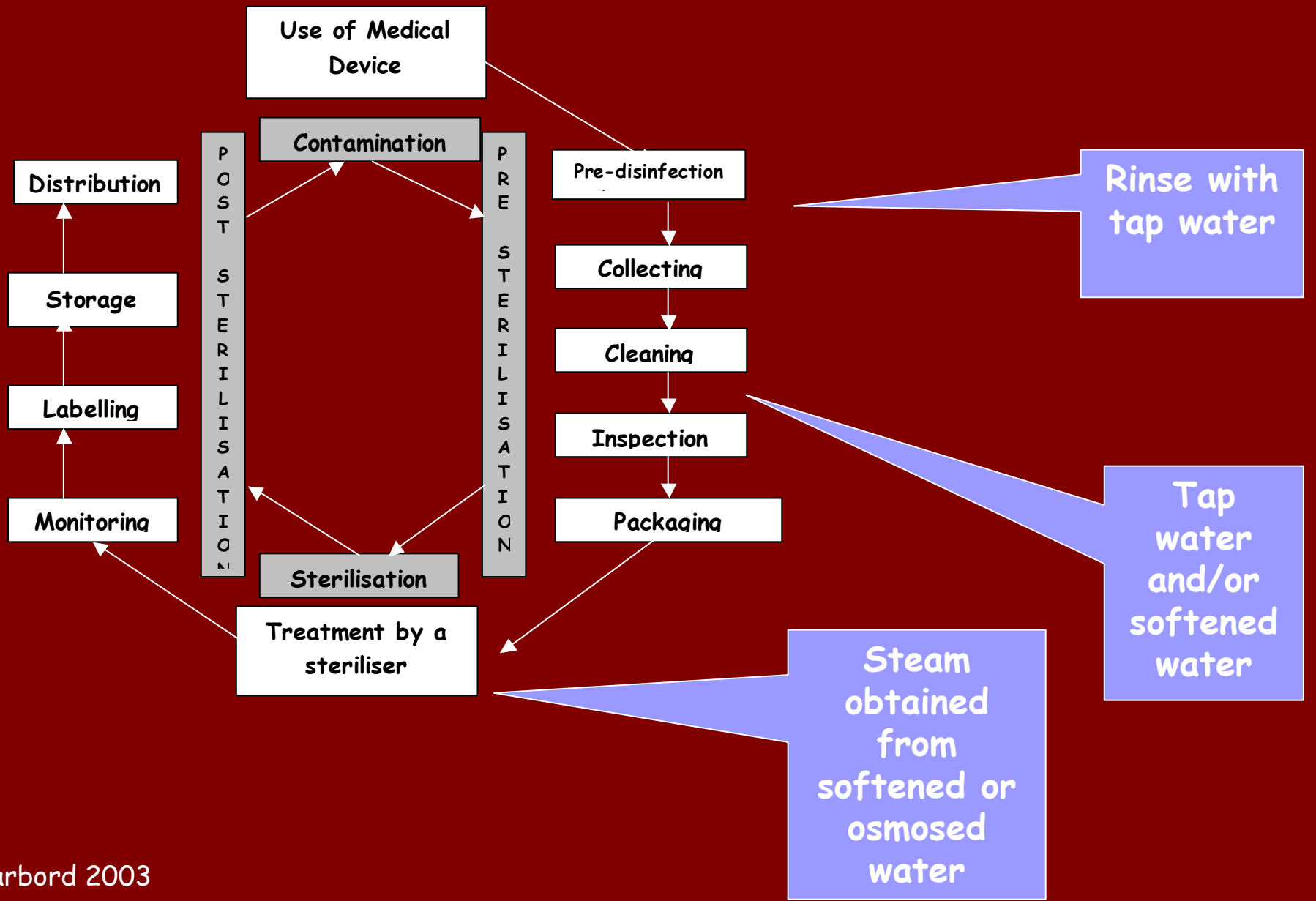
# BACTERIAL ENDOTOXINS or Lipopolysaccharides

- Constituents of the cell wall of Gram-negative bacteria
  - Negatively charged
  - Heat resistant
  - Realeased by bacterial multiplication or destruction
  - Specific interaction with immunity cells
- Pyrogenic
- Cause the septic shock : hypotension, circulatory problems, death

  → Are concerned : lung, CNS, liver, kidney …



# Where could endotoxins be found ?




Darbord 2003

# Endotoxinic contamination by water ?

- Martin et Dailey : heavily contaminated generators of bench top sterilisers (1158 IU/mL) ➔ possible transmission to the instruments ? *(BR. Dent.J. 2001)*
- CRDH : contamination of instruments by heavily contaminated rinse water (1998 IU/mL)*(Biomed.Instrum.Technol. 2004)*
- Holland : diffuse lamellar keratitis, contaminated instruments, evidence of the steriliser role (water in the generator) *(Ophtalmology 2000)*
- MAUDE : diffuse lamellar keratitis, instruments batch in cause (25 to 68 IU/instrument) and not water (0,88 IU/mL) *(Biomed.Instrum.Technol. 2004)*
- Whitby : low water concentration (5,3 IU/mL) and very low transmission of endotoxins by steam (<0,5 IU/mL) *(J. Refract.Surg. 2002)*

***➔ The transmission of the endotoxin contamination to instruments by steam is improbable***

# Measurement of endotoxins

- Limulus Amoebocyte lysates (LAL)
  - Principle Reaction : enzymatic chain➔ coagulation, turbidimetrie or chromogenic
    - Achievement : bleeding of Limulus and collection of the amoebocytes contents
    - substrate release
  - Sensitivity : 0,005 to 50 IU/mL
  - Technical constraints : delicate + liquid medium
- Application to (new) surgical instruments : recovery and measurement
  - FDA guideline (1983); AAMI (2004): 20 IU/instrument
  - Literature : Twohy, Ross, Novitsky, Rolansky, Berzofsky and Ragab

    ➔ Settling the recovery method

# Preliminary validations

- Validation of the recovery of inoculated endotoxins on instruments method :
  - Preparation of an endotoxin suspension 40 000 IU/mL
  - Depyrogenation of instruments : nine knee prosthesis LEPINE made of stainless steel, 316 LVM quality
  - Inoculation in batches of three at a rate of 4 000 IU for each device (10*10µL)
  - Drying at 37°C for 2h
  - Recovery by immersion in LAL quality water, then sonication for 25 min and shake for 5 min on the vortex
  - LAL measurement by kinetic chromogenic method
  - ***➔ Recovery rate of endotoxins on the instruments: 8%***

# Preliminary measurements

| Goals | Method | Samples | Conclusions |
|---|---|---|---|
| Evaluation of the endotoxinic contamination of the instruments after cleaning in a WD | Endotoxins recovery as described before and LAL measurement | 5 ENT instruments (microscissors, microforceps, gouge) | < 3,125 IU/instrument (< sensitivity threshold) < FDA limit (20 IU) |
| Endotoxin quantity in water | LAL measurement | 8 samples | In UI/mL : Tap water 5 Boiled water 0,07 Softened water 1 Demineralised water 0,02 |

# Inactivation kinetic of endotoxins by saturated steam

| Goal | Method | Samples | Conclusions |
|---|---|---|---|
| Evaluation of depyrogenation by steam of the instruments inoculated with known quantities | • Experimental Autoclave<br>• Inoculated instruments<br>• Recovery<br>• LAL measurement | At 134°C :<br>• 4 : 3 min tests<br>• 4 : 18 min tests<br>• 4 : 30 min tests | Obtained reduction rate :<br>• 50%<br>• 91%<br>• 97% |

# Steam generator effect

| Goal | Method | Samples | Conclusions |
|---|---|---|---|
| Evaluation of depyrogenation by the water of the steam generator | • Experimental autoclave<br>• Water of the steam generator inoculated with known quantities<br>• Heating at 135°C<br>• Withdrawal of samples out of the generator after cooling<br>• LAL measurement | 8 samples | Obtained reduction rate :<br>After 15 min :<br>2,8101 log |


PASTEUR

# Steriliser effect

| Goals | Method | Samples | Conclusions |
|---|---|---|---|
| Evaluation of the ability of steam to carry endotoxins, and contaminate the instruments in the steriliser chamber | • Experimental autoclave<br>• Inoculated generator<br>• Depyro-genated instruments<br>• Release<br>• LAL measurement | 2 withdrawal out of the generator and 6 instruments | 125°C-20 min and 134°C-18 min cycles:<br>< 3,125 IU/instrument<br>(< limit threshold)<br>< FDA limit (20 IU) |

# Conclusions (1)

## Quote : FDA limit by instrument : 20 IU

| STERILISATION STAGE | ENDOTOXINS | | RECOVERED ENDOTOXINS |
|---|---|---|---|
| | MEASURED | SPIKED | |
| Tap water (manual washing) | 5 IU/mL | | |
| Softened water (WD) | 1 IU/mL | | |
| Instruments after washing | < 3,125 IU/instrument (< limit threshold) | | |

# Conclusions (2)

## Quote : FDA limit by instrument : 20 IU

| STERILISATION STAGE | ENDOTOXINS | | RECOVERED ENDOTOXINS |
|---|---|---|---|
| | FOUND | SPIKED | |
| Inactivation by steam (of the instruments) : 18 min - 134°C | | 200 IU/ instrument (4 IU/mL) | 16 IU/instrument (0,32 IU/mL) |
| Inactivation in the steam generator | | 15 IU/mL | 0,02 IU/mL |
| Transmission of contamination from the generator to the instruments in the steriliser chamber | | 220 IU/mL (generator) | < 3,125 IU /instrument (< limit threshold) |
| TOTAL | **Water** 1 to 5 UI/mL<br>**Instruments** < 3,125 IU | | **Instruments** < 3,125 UI < limit threshold |

# Conclusions (3)

- This study, whose findings should be confirmed, tends to show that **there is no risk of endotoxin contamination of the instruments if the water used for the sterilisation process is contaminated**.

- **The results obtained by this research have no statistical pretension**. The reagents used for the measurements are tremendously expensive. The goal was to perform a first investigation in a field very little explorated yet, asking for questions on the quality of the process at the users.

# New works on the same topics have been published after ours

- First study : Kober P. « The implications of Endotoxins for sterilisation of surgical instruments » Zentr. Steril (2006), 14N°5, 372-373

| Type of water samples | Microbes | Endotoxins (EU/mL) |
|---|---|---|
| Last rinse water WD 1 | 0 | < 0,1 |
| Last rinse water WD 2 | 0 | 0, 426 |
| Demineralised water from storage tank for WD (85℃) | Coagulase – staph, 3/mL | 0,1705 |
| Demineralised water from storage tank (11 days after) | *P. putida, Pr. mirabilis, S. maltophilia, Flavobacteria* | 3, 275 |
| Water from storage container for steam generator, steriliser 1 | *Pr. Mirabilis, S. maltophilia, A. lwoffi* | 5,741 |
| Condensate, steriliser 1 | - | 0,4793 |
| Water from storage container for steam generator, steriliser 2 | *S. maltophilia, A. Lwoffi,* Gram negative bacilli | 1,204 |
| Condensate, steriliser 2 | - | 0,1467 |

Cycle 134℃ (134,4℃) – 5 min

# Conclusions of the Kober study :

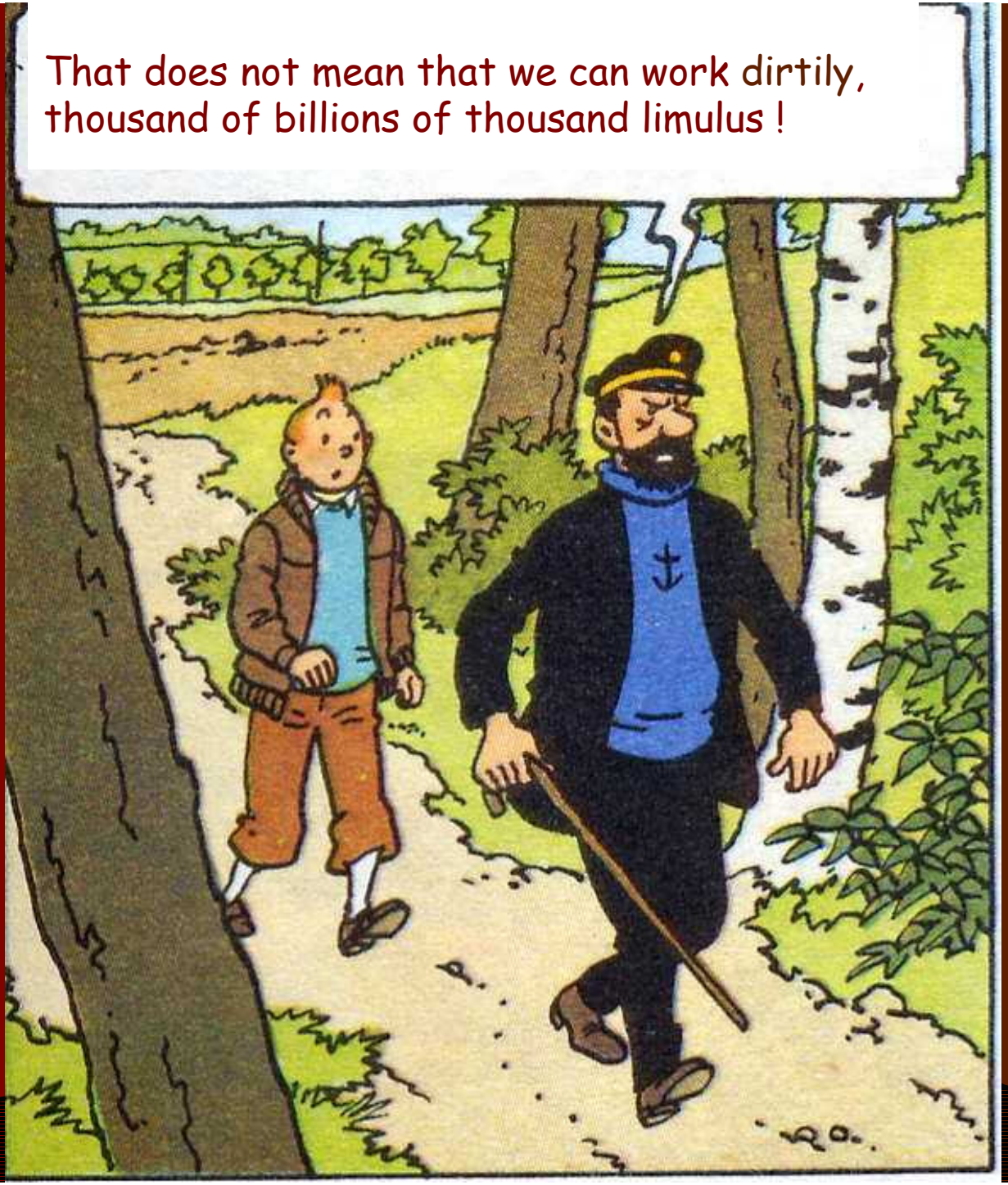
- The levels of endotoxin concentration are very low
- In large sterilisers, endotoxins are transported with the steam, and deposited on the instruments.
- The reduction from the feed water container to the condensate for the two sterilisers was by a factor of around 10.

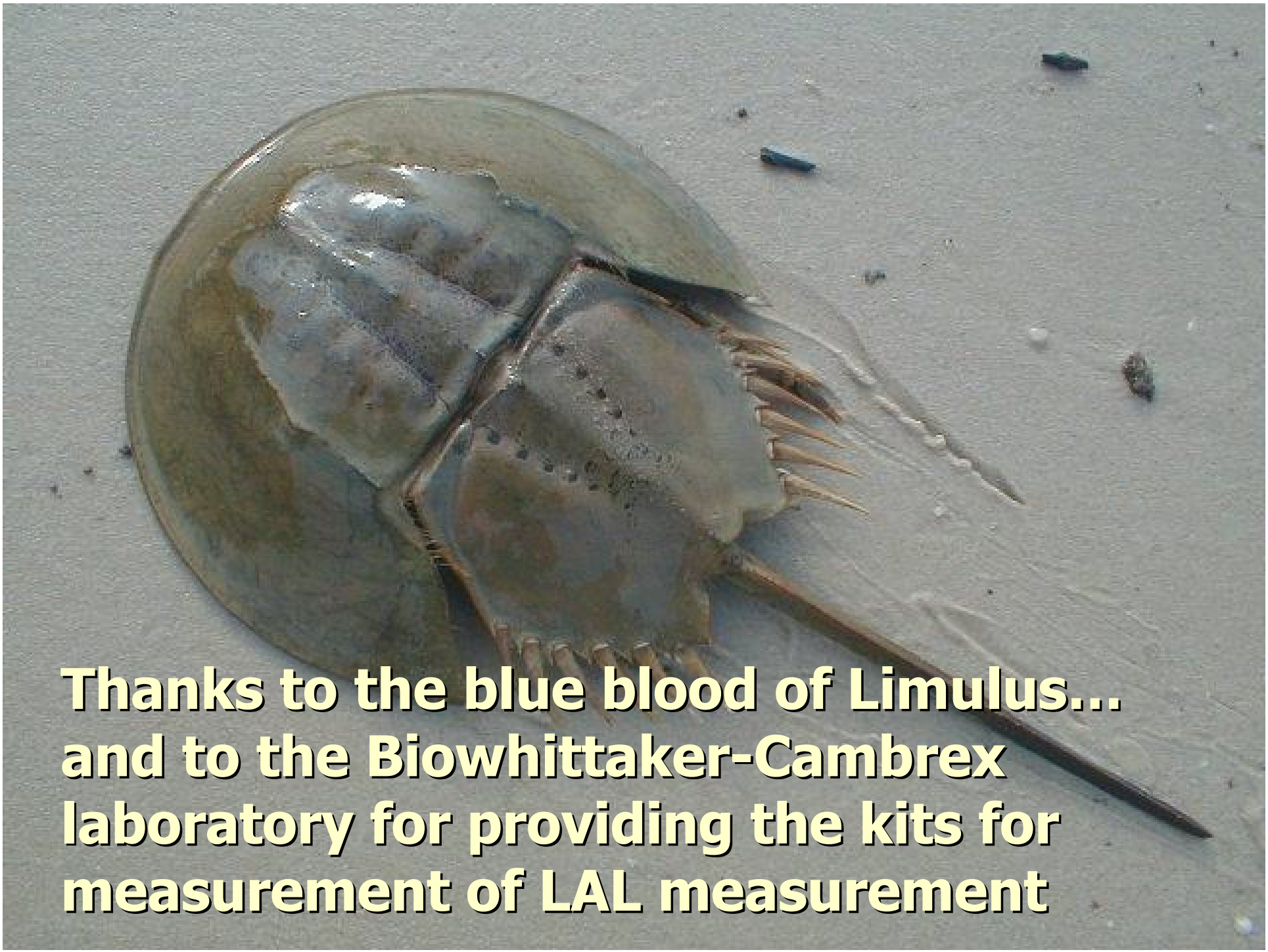
# Second study : Steeves A., Steeves R.M. « Endotoxin and reprocessing of medical devices »

Zentr Steril. (2006), 14N°5, 364-368

- Determine the effect of each reprocessing stage on instrument endotoxin level
- Investigate the effects on endotoxin levels when the three processing stages were carried out in sequence as is routinely done in the CSSD
- Sterilisation 134°C-3,5 min

- No endotoxin was detected on any of the instruments, inoculated or not, after washing and rinsing
- Staff handling the instruments in the cleanroom contaminated with endotoxin (0-1,35 EU)
- Chemical detergent, mechanical and dilution effects of the washing removes all endotoxinic activity.
- The endotoxin detected on instruments rinsed in softened water are the result of rinse water evaporating on the instruments during the drying cycle.


That does not mean that we can work dirtily, thousand of billions of thousand limulus !


Thanks to the blue blood of Limulus…
and to the Biowhittaker-Cambrex
laboratory for providing the kits for
measurement of LAL measurement