PAH Endotoxin Testing Service

David A Vesey, PhD

Centre for Kidney Disease Research
Translational Research Institute
Princess Alexandra Hospital
Outline

• What is endotoxin?
• Why test for endotoxin?
• How we test for endotoxin?
• PAH endotoxin testing service
What are we measuring?

- Endotoxin = Lipopolysaccharide (LPS)
- Integral part of outer cell membrane of all GNB
- Released on death of GNB
- Not a pyrogen assay - other potential bacterial pyrogens besides LPS
- One GNB = 3 million LPS molecules
- Endotoxin units 1EU~100pg endotoxin
Structure of gram-negative bacterial LPS

- **O-Antigen**
- **Outer**
- **Inner**
- **Core**

**Cell exterior**
- Embedded in membrane
- Outer bacterial membrane
- Lipid A

**Cell interior**
LPS characteristics

- Monomers → aggregates (-ve charge) (10kDa) (1000KDa)
- Absorbs to hydrophobic/cationic materials
- Inactivated by heat >200°C > 30mins
- Ultrafiltration
Why test for endotoxin

- Patients exposed to large amounts of water
- Water systems potential to harbour bacteria
- Bacterial products can potentially enter the circulation including endotoxin
- Endotoxin can induce cytokine production by monocytes/macrophages/other cells (IL-6/TNFα)
- Dialysis patients have low grade chronic inflammation → morbidity/mortality
- Better patient outcome with ultra pure dialysate (<0.03EU/ml) ↓ atherosclerosis, ↓ vascular calcification, ↓ anaemia

How to test for endotoxin?

- 1920s
- Rabbit pyrogen assay
- IV injection
- Temp↑ 0.5°C
- < 0.5EU/ml
- Time
- Qualitative
**Limulus amoebocyte lysate (LAL) assay (FDA approved in 1983)**

**Frederik Bang, MD 1956**

- Limulus polyphemus
- Amoebocytes
  - Endotoxin
- Clotting

Limulus amoebocyte lysate (LAL) assay is a method used to detect endotoxins, which are toxic substances produced by Gram-negative bacteria. It was developed by Frederik Bang in 1956 and was FDA approved in 1983.
• Aqueous extract of blood cells (Amoebocytes)
• Limulus Amoebocyte Lysate Assayc
  • Charles River Endosafe
  • Associates of Cape Cod
  • Lonza
How do we test for endotoxin?

Kinetic LAL assay (Chromogenic, turbidimetric)

Gel clot LAL assay

Endosafe PTS/MCS
Recommended maximum levels of microbiological contaminants in fluids used for haemodialysis

<table>
<thead>
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<th>AAMI USA</th>
<th>ERA-EDTA</th>
<th>JSDT Japan</th>
<th>ISN Italian</th>
<th>CARI Australia</th>
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<td><strong>Water for dialysis</strong></td>
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<td><strong>Ultrapure dialysate</strong></td>
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<tr>
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</table>

*Frequency of testing
PAH endotoxin testing service 1

- Kinetic chromogenic assay (CR Endosafe)
- 0.005 – 5EU/ml
- Submission form
- Courier at 4°C
- Arrive 10am – 4pm weekdays
- Wednesday/Thursday (most testing)
- Results within 48h - fax/e-mail/mail
- Cost $60/sample (currently)
PAH endotoxin testing service 2

- When: ASAP after taken, but store at 4°C
- Dialysate / RO water / post carbon / other
- Inhibition/enhancement
  - Enzymatic assay subject to interference
  - Can get inhibition/enhancement
  - NaCl, Bicarb, Acetic acid, bacterial products…
  - May need to dilute samples to overcome interference = reduced sensitivity
  - Need positive product control (PPC) for each sample
Sample collection

- Borosilicate glass container
- Sterile certified endotoxin free
  - > $15 each
Sample collection 2

- Sterile polystyrene tube
- Certified endotoxin free (<0.06EU/ml)
- 35c/tube
- Interpath services, BD
- I can send you details
Conclusions

- Endotoxin = Lipopolysaccharide (LPS)
- LAL assay – currently best assay available.
- very sensitive
- but expensive
- various formats
- point of treatment testing available with hand held devices – 15mins/sample
- but cartridges are currently expensive