



Australian Government

Department of Health

Therapeutic Goods Administration

Common mistakes and misconceptions with endotoxin testing

Endotoxin Testing – Doing the right thing ?

Australian Society for Microbiology
Cosmetics and Pharmaceuticals Special Interest Group

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24 July 2019

TGA Health Safety
Regulation



Presentation Plan

- Regulation of bacterial endotoxins
- Brief history of pyrogens, endotoxins – testing and regulation
- TGA involvement with validation, training, operator qualification
- Where we were then ... and where we are now
- Some examples of some mistakes and misconceptions
- Reassurance that you probably already are doing the right thing



Therapeutic Goods Administration





Immunobiology Section

Role in bacterial endotoxin testing and evaluation aspects

- Within the TGA Laboratories Branch
- Post-market monitoring of vaccine quality
- Endotoxin
- Evaluations
 - basically limited to bacterial endotoxin specification, method, validation/qualification
 - remembering that it is a pharmacopoeial test
 - so everyone should be **doing the right thing** ???
- Post-market surveillance testing
 - test some vaccines on a regular basis
 - test complaint samples
- Support to inspection team
 - occasionally



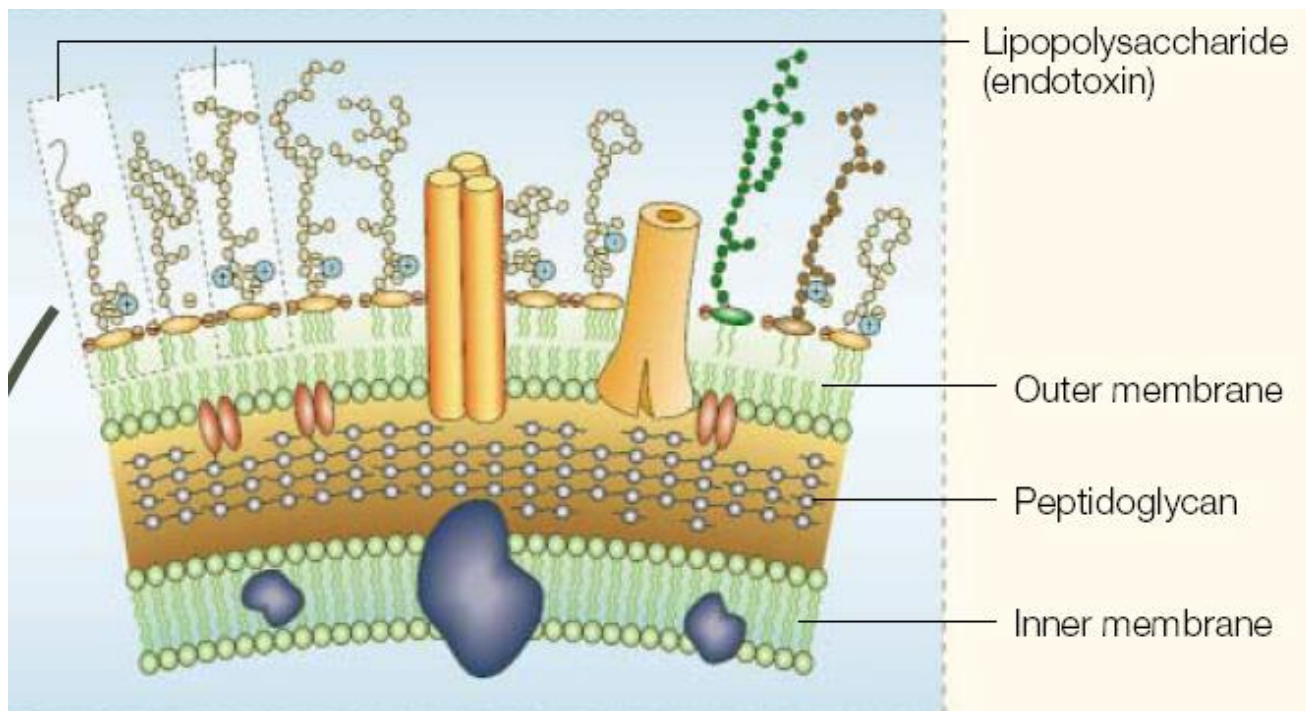
Pyrogens

What are pyrogens

- Substances that can cause a rise in body temperature when placed into contact with the blood stream
- Overwhelmingly the source of contaminating pyrogens in medicines and medical device industries is bacterial endotoxins from Gram negative bacteria
- This is the lipopolysaccharide from the outer membrane
- The benefit should outweigh the risk



Bacterial Endotoxins



Gram negative bacterial cell membrane

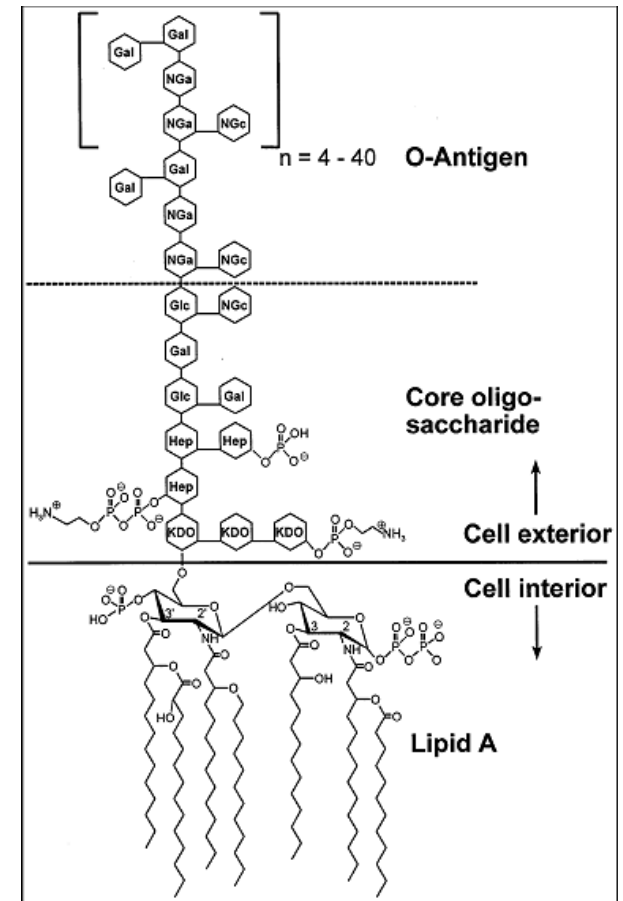
Beutler and Rietschel, Nature Rev Immunol 3, 169-179



Bacterial Endotoxins

Lipopolysaccharides

- Component of cell membrane of Gram negative bacteria
- Causes wide range of inflammatory responses – pyrogenic (fever), shock
- Lipid A section causes most of the biological activity





Testing for Pyrogens

Rabbits

- Rabbit Pyrogen Test – basically started in the 1920's, but not a pharmacopoeial test until the 1940's
- Measures rise in rabbit's temperature before and after administration
- Difficult and expensive





Testing for Pyrogens

Horseshoe Crabs

- Existed for 400 million years
- Endotoxin from bacteria causes the blood to coagulate as a defence mechanism
- Studied by Levin & Bang in 1950's & 1960's developing blood cell lysates ...
- *Limulus* Amoebocyte Lysate (LAL) has been used since the 1970's



https://www.msu.edu/~jaegeran/Andrea_Miehls_Photography



Bacterial Endotoxins

Assay Validation and Operator Training

- TGA Laboratories had previously been involved in running and attending training days for endotoxin testing
- Always conducted in conjunction with one of the reagent supply companies
- This was quite a long time ago
- When asked to give an update on operator training and test validation – there is not much TGA Laboratories need to add, except reassurance



Bacterial Endotoxins

Where were we then

- Replacement of the RPT and the introduction of BET was a big change
- For manufacturers and for regulatory bodies
- FDA Guidelines released 1987, updated 1997 ... provided some clarity
- Different pharmacopoeia were moving quite independently
- Moving from a well known test is always difficult
- Especially when it involves a patient safety parameter





Bacterial Endotoxins

Where were we then

- From Rabbit Pyrogen Testing to Bacterial Endotoxin Testing was a giant leap



Bacterial Endotoxins

Where were we then

- Since then all the steps have been smaller



- Gel clot ... plate assays ... turbidimetric ... chromogenic ... kinetic ... cartridges ... recombinant factor C ... even monocyte activation testing



Bacterial Endotoxins

Where are we now

- Harmonised pharmacopoeia
- Easier to understand
- Ph. Eur. has a Guideline for using the Test for Bacterial Endotoxins (5.1.10)
- FDA Guidelines replaced by Pyrogen and Endotoxins Testing: Q&A 2012
- Simply a more familiar test
- Manufacturing is also in a much better place
- **Everything you need to about training and operator validation for bacterial endotoxin testing should follow the same processes as all other testing ... in conjunction with the information in the pharmacopoeia**
2.6.14, 5.1.10, <85>, FDA Q&A



Bacterial Endotoxins

Where are we now ... and what this means

- Endotoxin testing is a pharmacopoeial test
- The field of reagent manufacturers is limited - and very committed to helping
- Think about what each part of the process means and the proper way to do it
- Care that you are **doing the right thing**
- Small steps are great but occasionally we are taken out of our comfort zone
cartridges ... monocyte activation testing ... recombinant factor C ...
... low endotoxin recovery
- **Every step has required sensible thinking to move on**



Case studies

Misconception - Pooling samples

- Combining samples for testing – most often 3 samples
- Sampling – **old** FDA Guidelines recommended at least one sample be taken from the beginning, middle and end of production run
- Pooling reduces the amount of testing, while still testing these samples
- But BET is not the same as other content tests – cannot be averaged
- BET should represent a position whereby ANY or EVERY single sample would meet the specification



Case studies

Pooling samples

- BET is looking for a contaminant that should not be there, but could be there in differing amounts in different samples
- Pooling is actually diluting out one sample with another
- When you test, the raw result is multiplied by the dilution that you used
- If you pool samples, this extra 'dilution' MUST be included
- Maximum Valid Dilution (MVD) – if you test at this dilution, you are testing at the very limit of the assay



Case studies

Pooling samples

- Many companies, particularly overseas manufacturers, test at “MVD/2”
- This means that they are effectively carrying out the test at half of the limit
- A pool of 3 (or more) samples cannot be tested at MVD/2
- As per the US FDA Guidance for Industry – Pyrogen and Endotoxins Testing: Questions and Answers (June 2012)
- If you pool samples PLEASE include this extra dilution in assay calculations



Case studies

Misconceptions and mistakes - Product X

- Samples came to TGA Laboratories as a complaint
- Overseas manufacturer
- The testing has to be conducted closer to the limit (near the MVD)
- Tests in our laboratory gave out of specification endotoxin results
- Why were we getting results higher than the limit for some of the samples we were testing



Case studies

Product X

- Way we tested was probably different to the way most other labs test
- We try to take aliquots the same way that the product is used clinically ...
- Some vials had residual water under the aluminium seals
- Stoppers were 'rougher' than comparator products
- Product is terminally sterilised using a rotary autoclave



Case studies

Product X

- Investigation led to a TGA inspection team being sent to the manufacturer
- Looked at the above factors
- Were there any other problems ?
- No single cause identified
- What were some of the solutions that fixed the issue ?
 - water ... stoppers ... culture ...
 - smart, clear thinking ... caring about **doing the right thing** ...



Case studies

Glutathione from a Compounding Pharmacy

- Adverse event report came in from NSW Health that some patients had experienced symptoms after receiving intravenous infusions of glutathione
- These were filter sterilised infusions prepared for individual patients
- TGA Laboratories was asked to conduct some testing
- Working with the Chemistry and Microbiology Sections
- Tested various infusion vials that had been prepared for different patients, and samples of glutathione powder used to make the infusion
- All 'batches' of infusion were sterile but some had high endotoxin levels



Case studies

Glutathione from a Compounding Pharmacy

- After requesting further information from the pharmacy ...
- The pharmacy had used a new supplier of glutathione powder
- The batch of glutathione powder from the new supplier also had high endotoxin levels and may not have been the correct quality
 - noting that glutathione can also be taken orally
- Importance of having a proper system for checking raw material suppliers
- Importance of thinking about the process you are undertaking
- Importance of caring that you are **doing the right thing**



Summary

Endotoxin testing - Assay qualification & Operator training

- All in pharmacopoeia
- Reagent manufacturers are a great source of information
- Adhere to standards and guidelines that apply to the environment you work under... whether it be GMP, ISO standards, hospital guidelines, medical device standards orders, etc.
- Think about what you want to do and trust yourselves and your processes
- If you care about **doing the right thing** ... you will



Questions



http://www.mnh.si.edu/exhibits/natures_best_2006/gallery/horseshoecrabs.jpg



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