Pharma&Biotech

Medical Devices: Guidelines for Performing the Bacterial Endotoxins Test (BET)

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Lonza

60-Minute Agenda

- Regulations
- What constitutes a device?
- Extraction procedure
- Establishing a sampling plan
- Calculating Endotoxin Release Limits (ERL)
- Suitability testing
- Special cases
- Q&A session

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Endotoxin Testing Regulations for Medical Devices

- The regulations regarding the final release testing for medical devices vary between the United States and Europe
- In the United States, the FDA enforces the monograph contained in USP Chapter <161>, which will be described in subsequent slides
- In Europe, regulations regarding final release testing of batches of medical devices are less well defined and are a ‘risk assessment’ based system

Stent

Information Sources for Medical Device Testing in General

- USP Chapter <161> (Amended)
- ANSI/AAMI ST72:2011*
- LAL vendors information sheets

* AAMI stands for the Association for the Advancement of Medical Instrumentation

Medical Device Testing – Principle

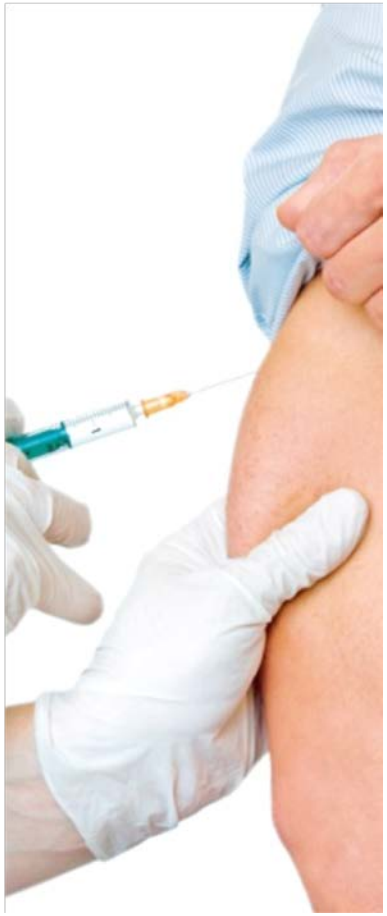
- In order to test medical devices for endotoxin, the endotoxin will have to be washed off (extracted/rinsed) from the surface of the device
- This means that the actual sample tested for endotoxin is the extracting solution
- This principle will apply irrespective of US or European regulations and at the current time, only the USP monograph provides clear guidance as to how the extraction should be carried out

Endotoxin Testing Procedure

- Any of the approved Bacterial Endotoxins Test (BET) methods can be used to test the extract
- The extract must be tested in duplicate with a Positive Product Control (PPC) also run in duplicate
- The result will be reported as a pass or fail, according to whether or not the test result is below or exceeds the calculated endotoxin release limit for the devices that are being tested

Update to Chapter <161>

- USP has released an amendment to Chapter <161>, effective from August 1, 2015
- The amendment contains several changes to the previous text, the main changes have been highlighted in later slides
- We recommend you obtain a copy and read it through as all changes, especially minor ones, may not be shown in this presentation
- Important changes relate to:
  - Product unit descriptions
  - Sampling
  - Suitability testing
- Key elements such as endotoxin limits are unchanged



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What Constitutes a Device?

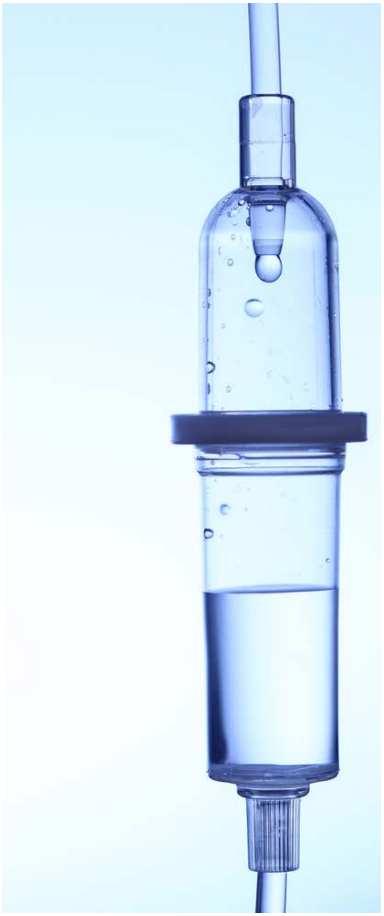
- The best general description is contained in the AAMI document ST72:2011:

  "Products that have direct or indirect intravascular, intra-lymphatic, or intrathecal contact, or have the potential for similar systemic exposure shall be evaluated for the presence of endotoxin."

- The application of any non-pyrogenic product label statement or claim also requires explicit substantiation in the form of endotoxin testing

New USP <161> – Examples for Medical Devices

- Fluid pathways of catheters and administration sets such as:
  - Solution administration sets
  - Extension sets
  - Transfer sets
  - Blood administration sets
  - Intravenous catheters
  - Implants
  - Extracorporeal oxygenator tubings
  - Dialysis tubing
  - Intramuscular drug delivery catheters
  - Transfusion and infusion assemblies
- Liquid medical devices such as dialysate



New USP <161> – Examples for Medical Devices (ctd.)

- Implantable medical devices such as heart valves and vascular grafts, other medical devices with a non-pyrogenic claim that may come into contact with blood or cerebrospinal fluid
- Gels with a non-pyrogenic claim including demineralized bone matrices and drug delivery systems

Complex Devices

- Today, there are many complex devices that need careful consideration when it comes to endotoxin testing
- Manufacturers should ensure that all fluid paths that could come into contact with systemic fluids are included in the extraction process
- If there are ‘non-contact’ fluid pathways in the same device, manufacturers should make sure there is documentary evidence of the lack of contact with systemic fluids

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New USP <161> – Extraction Procedure

- The description for the extraction process is as follows
  - *“The standard extraction method is to soak or immerse the device or flush the fluid pathway with extracting fluid that has been heated to 37±1.0°C, keeping the extracting fluid in contact with the relevant surface(s) for not less than one hour”*
  - *“Alternate extraction or rinsing methods may be used, but must be demonstrated to be equivalent or better than the standard method”*

Extraction Procedure

- The statement from the monograph makes it clear that the extracting fluid must be heated to 37°C but it does not indicate that it has to be maintained at 37°C for the hour. This implies that the extracting fluid can be allowed to cool over the hour to room temperature
- This is a practical benefit for larger devices and avoids the need for warm-air incubators
- Extracts for medical device testing are typically pooled according to the limits described in subsequent slides

Extraction Materials

- The normal extracting fluid would be LAL Reagent Water but it is also possible to use other fluids provided that can be shown to be non-interfering
- Extraction vessels and containers should be free from endotoxin and tested to ensure there are no interfering factors. Glass or polystyrene is generally recommended
- Any ancillary materials needed for the process (such as tongs, clips, etc.) should be stainless steel and depyrogenated

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Sampling and Number of Devices – Old

- When following USP <161> medical device manufacturers are required to test a number of devices per batch calculated as follows:
  - If the manufacturing batch size is <100 items, a minimum of 3 pieces should be subject to extraction and testing
  - If the batch size is >100 items, 3%* of the batch up to a maximum of 10 items should be subject to extraction and testing
- All batches must be tested

* Rounded up to the nearest whole number

New USP <161> – Selection of Devices for Testing

- The largest change in the text deals with how product units are defined and how the item to be tested is classified

Product Unit	Item for Testing
Individual medical device in primary package where each medical device is used individually in clinical practice	Individual medical device
Set of components in a primary package where components are assembled as a product and are used together in clinical practice	Combination of components
Number of identical medical devices in one primary package where each medical device is used independently in clinical practice	Single medical device taken from the primary package
Kit of procedure-related medical devices where each medical device is used independently in clinical practice and where each medical device may have a different endotoxin limit	Each type of medical device that has a non-pyrogenic claim, or all items together

New USP <161> – Sampling

- The sampling requirement changes from the old text shown previously to the text below:
  - Sample
    - A sample for end-product testing must be in its final configuration and packaging, including all component parts that make up the final medical device
  - Representative sampling
    - A representative sample is a sample plan that is created and justified based on the assumption that the manufacturing process is validated and in a state of control

New USP <161> – Sampling (ctd.)

- Sample size
  - The number of devices chosen for routine testing is dependent on the size of the lot, level of control, statistical considerations, and historical performance. In most cases, each lot of product must be tested using an appropriate number of samples, not more than 10, taken at random to represent the quality of the lot
  - Alternate sampling plans that utilize small sample sizes or do not test each lot of product must be clearly defined and must be supported/justified by a robust risk assessment
- Pre-post sterilization sample
  - When qualifying a BET assay for pre-sterilization samples, equivalency in test results must be shown with post-sterilization samples to ensure that sterilization and post-sterilization handling have no adverse impact on the accuracy of the test result. Pre-sterilization testing is inappropriate for products that support microbial growth

European Test Requirements: Number of Devices & Testing Frequency

- In the case of the current European regulations, both the number of devices that should be tested and the frequency of testing is a decision for the manufacturer based on risk assessment
- This is a rather ambiguous methodology, as it can be difficult to assess potential risk and establish a suitable testing regime
- For this reason and also because of export requirements, most manufacturers adhere to the testing procedures described in USP <161>

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Endotoxin Release Limits for Medical Devices

- Endotoxin release limits for medical devices are described in USP Chapter <161> and are fixed values (the constant K) as follows:
  - For medical devices that will not contact cerebrospinal fluid:

    K = 20 EU/device
  - For medical devices that may come into contact with cerebrospinal fluid:

    K = 2.15 EU/device

Endotoxin Release Limit for Extracting Solutions

- There is the potential for considerable variability in endotoxin content of the extract due to the volume of water required to wash the devices of differing sizes and the variable number of devices that need to be tested
- As a result, the ERL for extracting solutions is calculated from a formula that compensates for both variables
- Using a formula compensates for the fact that it may be necessary to use, for example, 40 ml of water to wash a small device such as a syringe, and perhaps 500 ml to wash a larger device such as a pacemaker

Extract Pooling

- There is an important difference in the testing of medical devices compared to parenteral products in that manufacturers are permitted to pool the extracts before testing
- As pooling could potentially hide endotoxin on an individual device due to the dilution effect, the formula used to calculate the ERL compensates both for the number of devices tested per batch and the total volume of water used in the extraction process

Calculating the ERL for Extracting Solutions

- The formula used is: $ERL = \frac{K \times N}{V}$

- Where
  - K = the endotoxin limit per device (20 EU or 2.15 EU)
  - N = the number of devices tested
  - V = the total volume of water used to extract the endotoxin from the number of devices (N) above

ERL Calculation Example

Example

Three large devices that will not contact cerebrospinal fluid, are each washed using an extraction volume of 300ml per device:

$$\mathrm{ERL} = \frac{\mathrm{K\,x\,N}}{\mathrm{V}}$$

$$\mathrm{ERL} = \frac{20\ \mathrm{EU}\ \mathrm{x}\,3}{900\ \mathrm{ml}}$$

$$\mathrm{ERL} = 0.06\ \mathrm{EU/ml}$$

Note: Medical devices can also be extracted and tested without pooling but the advantages of pooling are lost.

Comment on Device Limits

- It should be noted that there is a good deal of additional patient safety with the current medical device endotoxin limits
- For example, let's take a situation where 10 devices are tested, and only one of the ten devices is contaminated with the other nine free of endotoxin
  - In such a case, the maximum 'dose' of endotoxin the patient could receive from a single contaminated device would be <200 EU
  - This value is around 2/3 of what would be allowed for a parenteral injection

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New USP <161> – Suitability Testing

- There is now a requirement for suitability testing for each product or product family
- This is essentially similar to the test for interfering factors described in USP <85> for injectable drugs
- The manufacturer is required to determine the number of batches needed for suitability testing based on demonstrable process control
- Suitability testing establishes whether or not additional dilution of the extract (up to the Maximum Valid Dilution) is necessary to overcome any inherent interference

The Maximum Valid Dilution (MVD) and Medical Devices

- As the ERL obtained from the calculation (K x N)/V is in EU/ml, it is easy to obtain the MVD for a medical device extract
- The MVD is simply the ERL divided by lambda (λ)
- Lambda (λ) is either:
  - The labelled lysate sensitivity for gel clot assays

    or
  - The value of the lowest standard used in the assay for kinetic or endpoint quantitative methods
- For example, if the calculated ERL for the device extract is 1.0 EU, the MVD for the kinetic chromogenic method (lowest standard 0.005 EU/ml) would be 1:200

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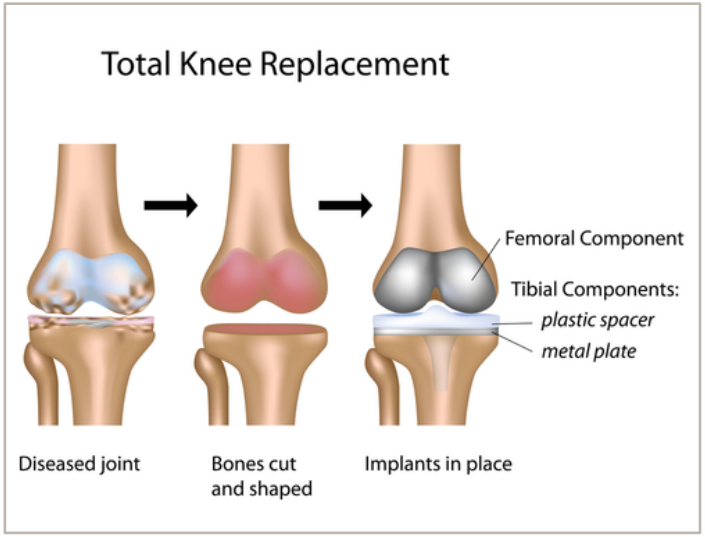
Complex Devices

- When working with complex devices, care must be taken to ensure that all the surfaces are accounted for
- For the example on the right, a single extraction would be needed for the femoral component, the plastic spacer and the metal plate, as these form the completed device
- There is no limit to the volume of water that can be used for the extraction, as this is accounted for by the (K x N)/V equation
- The only issue is that the ERL may well be a low value due to the high volume of water needed



Wound Dressings

- Wound dressings are classed as medical devices, but no endotoxin limit should apply, since they are not implanted
- They are instead considered to be topicals

Artificial Skin

- Artificial Skin (AS) is not regarded as a medical device
- Instead, AS is regarded as a combination product (biologic/drug) and therefore endotoxin testing is mandatory
- Due to the physical and biological properties of AS, a special procedure is needed for testing
- This procedure is not documented in any of the pharmacopeias but the next slide illustrates a proposed methodology that has been accepted by the FDA

Endotoxin Testing for Artificial Skin

- Due to the physical nature of AS, they cannot be tested using the normal procedures for injectable drugs
- It is necessary to test an ‘extract’ in a similar manner to that used for medical devices
- In this case, the growth/maintenance media that is used to store and transport the skin is tested for endotoxin
- The ‘extraction’ is regarded by the FDA as being more severe than the medical device requirements as the skin will be suspended in the media for a more prolonged period

ERL and Interference Testing for Artificial Skin

- In our experience, the FDA allowed the use of the 20 EU/device ERL for the testing of the suspension media
- The extract will need to be tested for interference following the same procedure as described in the BET monograph in USP chapter <85>
- This would mean calculating the ERL using the (K x N)/V equation where:
  - N = 1
  - V is the volume of the suspending media

Endotoxin Testing for Artificial Skin – Regulatory Considerations

- The MVD can then be calculated from ERL/λ and a screening protocol created to find a suitable dilution for routine testing
- We would recommend that any company developing a protocol for special products such as artificial skin should have it reviewed by their local regulatory agency before implementation
- Checking with the regulators in special cases like this is always advisable, as they may have some specific concerns that need to be addressed

Conclusions

- Medical devices are tested for endotoxin by washing the endotoxin off of the device with LAL Reagent Water
- Due to variability in the size of devices and their cost, manufacturers are allowed to vary the number of devices and the volume of LRW used
- These variables are compensated by calculating the ERL for device extracts using a formula described in the monograph in Chapter <161> of the USP
- Devices must be tested with a PPC, to ensure no interfering factors are present
- The extract can be diluted if interference is detected, in accordance with the MVD
- Artificial skin is regarded as a combination product and is likely to require special test procedures

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  - Validation and Regulatory Acceptance of Alternative Endotoxin Detection Methodologies

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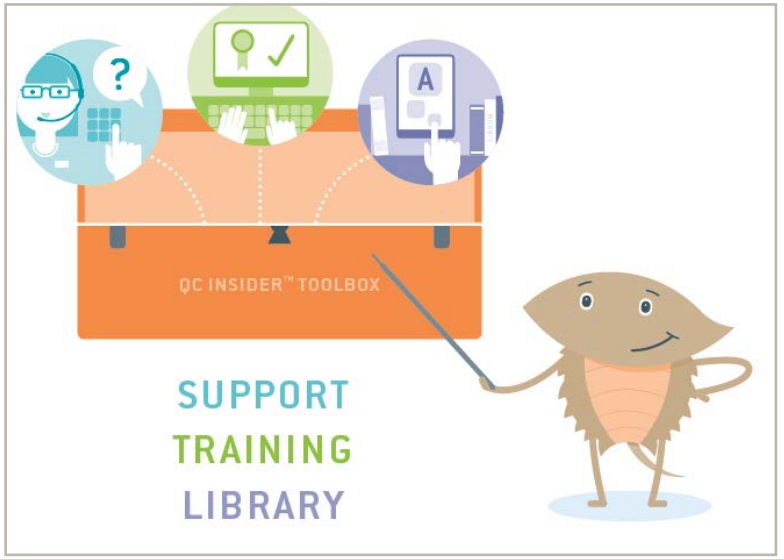

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