Rapid Sterility Testing

Miriam Guest
Associate Principal Microbiologist,
New Modalities & Parenteral Development, Pharmaceutical Technology & Development, Operations, AstraZeneca, Macclesfield UK
ATP bioluminescent growth based test selected

- Technology evaluation performed to assess portfolio and challenges;
  - 14 days lead time on results for traditional methodology
  - Holistic sterility assurance strategy considered, e.g. environmental batch release docs, visual inspection considered
  - Hands on time and sample preparation
  - Regulatory acceptance of techniques
- Able to test aqueous and solid products
- Accepted for this application
- Potential use for other development activities
- Potential application for other activities in commercial Ops labs
- Able to test complex sample matrices
- Inform of any issues faster and be able to respond
  - Faster access to medicines for our patients
• Review data
• Risk assessment for traditional sterility testing
• Risk assessment for application of ATP Bioluminescence for sterility testing
• To mitigate risks, work packages were identified;
  • Build method verification data
  • Method validation packages
  • Training packages and assessments
• Considered both product specific and non-product related risks
  • manufacturing process, manufacturing sites, variability in available consumables, method performance, formulation
• Use of surrogates to build data where material may be scarce
Review & Risk Assessment

- Review data
- Risk assessment for traditional sterility testing
- Risk assessment for application of ATP Bioluminescence for sterility testing
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- Use of surrogates to build data where material may be scarce
Tech Transfer to Sites; Primary Verification Study to confirm method operates as expected. Method suitability aligned to traditional sterility test

Environmental Isolates Detection
Global review of pharmaceutical microbial IDs – focus on most frequently isolated organisms/groups balanced against data already available

Variability in consumables

Variability in performance

Product related factors
Worse case sample effects
Consider surrogates
Additional specificity data
Link suitability to traditional sterility test

Review and Risk Assessment

Method Equivalency is Demonstrated
Equivalency Study (vendor)

- Membrane filtration sample preparation and incubation
- Fluid Medium A
- Ruggedness criteria
  - Various lots of media
  - Various operators

Organism Panel

- Staphylococcus aureus
- Pseudomonas aeruginosa
- Clostridium sporogenes
- Bacillus subtilis
- Candida albicans
- Aspergillus brasiliensis

The following environmental isolates underwent stress challenges prior to inoculation
- Propionibacterium acnes
- Staphylococcus epidermidis
- Micrococcus luteus

Supplemented with additional environmental isolates
- Burholderia cepacia
- Methylobacterium extroquens
- Penicillium citrinum

Three inoculum levels for each organism
10CFU – 10 replicates
1CFU – 28 replicates
0.1CFU – 10 replicates

Pooled results from all 12 organisms at the 3 inoculum levels obtained sufficient statistical power for the testing of equivalence with traditional method.
The study demonstrates the ATP method is non-inferior to the Compendial Method.
The limit of detection was defined as 0.08CFU.
Determination of suitable surrogates

- Build data from knowledge active pharmaceutical ingredients behaviours in the presence of microorganisms
  - Bioburden method suitability data
- Challenge study data supporting storage or processing stages
- Formulation understanding and dose concentration range
- Application to product families
- Considerations for formulation buffers which are used across multiple products
- Relationship with filtration, including production filters and sterility test filters
- Use filter compatibility knowledge to select appropriate sterility test filter membranes
AstraZeneca performed a review of global data on the identification of isolates from pharmaceutical facilities; using data from microbial identification services and comparing with knowledge of AZ networks commonly isolated organisms. Supplemented data with the following panel of organisms, in a specificity verification study:

- **Micrococcus luteus** – most commonly isolated organism from clean rooms
- **Staphylococcus capitis** – additional GPC – majority of clean room isolates are GPCs
- **Bacillus altitudinis** - represent GPR – only one GPR in original equivalency study, but GPR feature in most frequently isolated organisms from pharmaceutical environment (at lower levels than GPCs)
- **Penicillium chrysogenum** - represent additional mould species – considered representative of worst case due to slow grower
- **Cutibacterium acnes** – represent slow growing organisms

### Absence of Product

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. luteus, S. capitis, Cutibacterium acnes</td>
<td>TSB &amp; FTM</td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>TSB</td>
</tr>
</tbody>
</table>

Three inoculum levels in absence of product; 10CFU, 1CFU, 0.1CFU

Performed in duplicate as verification of specificity study

### Presence of Surrogate product

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. luteus, B. altitudinis, S. capitis</td>
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</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>TSB</td>
</tr>
<tr>
<td>Cutibacterium acnes</td>
<td>FTM</td>
</tr>
</tbody>
</table>

On three lots of product, in duplicate; target 10CFU inoculum level

### Alternative products

Risk assess but include this panel as a “standard”

- <10CFU target inoculum & following “AZ Panel” plus compendial
  - M. luteus
  - S. capitis, Cutibacterium acnes – using FTM
  - Penicillium chrysogenum, B. altitudinis – using TSB

On three lots of product, in duplicate; target 10CFU inoculum level

- M. luteus, B. altitudinis, S. capitis – using TSB & FTM
- Penicillium chrysogenum – using TSB
- Cutibacterium acnes - using FTM

Performed in duplicate as verification of specificity study
Opening the testing window to build range of options

Understanding variability in consumables
- Nutrient media
- Rinse fluids
- Sample diluents
- Sterility Test Cannisters

Laboratory variability
- Sample handling prior to ATP read
- Time to result

Assessment of Product impact
- Building knowledge of product behaviour in the assay
### Nutrient Media Studies

<table>
<thead>
<tr>
<th>Vendor Supplied</th>
<th>Purchased media</th>
<th>In-house preparation</th>
<th>Rinse Fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Vendor supplied media used for equivalency study and majority of method validation.</td>
<td>• Purchased wide range of ready to use sterility testing media</td>
<td>• Greatest variability both within a batch of media and across batches</td>
<td>• Generated data from canisters using range of rinse fluids, suppliers, types, batches, in-house prep</td>
</tr>
<tr>
<td>• Mitigate risk of single supplier</td>
<td>• Background measured at different ages (including end of shelf life)</td>
<td>• Process improved with “new” vials vs. recycled</td>
<td>• No significant difference observed within the testing framework</td>
</tr>
<tr>
<td></td>
<td>• Observed intra and inter-batch variability</td>
<td>• Unlikely to use routinely</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• More background vs. Vendor supplied, however should a back-up be required</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quality Control Checks Outline</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Background monitoring of nutrient media, across suppliers

Background RLU levels from TSB different suppliers
Background monitoring of nutrient media, across suppliers
Within batch performance

Reading of background RLU of FTM Site comparison

- Site A
- Site B
Time to result studies nutrient media

Time to result, visual and ATP

- Used range of suppliers
- *Cutibacterium acnes*
- *Aspergillus brasiliensis, Penicillium chrysogenum*
- Target inoculum <10CFU
- Time to result by ATP bioluminescence always faster than visual detection
- Visual time to result varied with supplier of media

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum count (cfu)</th>
<th>Time to Result Visual</th>
<th>Time to Result ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus brasiliensis</em></td>
<td>2</td>
<td>Day 6</td>
<td>Day 6</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>12</td>
<td>72 hours or Day 6</td>
<td>72 hours</td>
</tr>
<tr>
<td><em>C. acnes</em></td>
<td>5</td>
<td>Day 5-11</td>
<td>24 hours</td>
</tr>
</tbody>
</table>
Sterility Test Canisters

Range commercially available

• Supplied with sample port preferred
• Different filter membrane types and cannister plastic for product compatibility
• Sample extraction when no port experimental programs executed to support

Background from Sterility Test Canisters

• Whilst the sample port is preferred, consistent results observed with other sterility test canisters
• Trialled different filter materials and different plastics.
• Broth calibrators are key for this assay, so parallel negative control is important
• FTM BC 800-3300 RLU background
• TSB BC 300-1200 RLU background
**Product Sample Effects**

**Worse-case assessments**

- Samples of product directly into the ATP bioluminescence assay
- No filtration steps
- Neat, diluted in the nutrient media and incubated at equivalent test temperatures
- Build library of knowledge

**Summary of Product Backgrounds**

<table>
<thead>
<tr>
<th>Product</th>
<th>Background RLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product A</td>
<td>990</td>
</tr>
<tr>
<td>Product B</td>
<td>2346</td>
</tr>
<tr>
<td>Product C</td>
<td>1065402</td>
</tr>
<tr>
<td>Product D</td>
<td>863</td>
</tr>
<tr>
<td>Product E</td>
<td>873</td>
</tr>
<tr>
<td>Product F</td>
<td>686</td>
</tr>
<tr>
<td>Product G</td>
<td>78</td>
</tr>
</tbody>
</table>
Overcoming Sample Effects

Sample effects

• Minimal interreference, proceed in method suitability with canisters (product limitation maybe scale down)

• Filtration experiments to see if the “effect can be retained by switching sterility test canister”

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unfiltered 1</th>
<th>Cellulose filtered</th>
<th>Unfiltered 2</th>
<th>PVDF filtered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 FTM dilution</td>
<td>NT</td>
<td>NT</td>
<td>131112</td>
<td>108412</td>
</tr>
<tr>
<td>1:100 FTM Dilution</td>
<td>28474</td>
<td>2971</td>
<td>7909</td>
<td>6297</td>
</tr>
</tbody>
</table>

Data showed the “sample effect” would likely be retained using cellulose filters, proceeded into method development with PVDF sterility test canisters. Demonstrating method suitability
Building data with minimal product

- Use of surrogate to build data at range of inoculums and full organism panel
- Product through canister, no rinse + nutrient media, incubate and read
- Scaled down worse-case sample effects
- 1/3 of all material including rinses/nutrient media and inoculums
- Method suitability aligned with USP<71>, including parallel ATP and visual reads
Turbid products

- Complex parenteral with emulsification step and polymer present
- Sub-culture steps
- Risk assessment of sub-culture steps to understand risk of traditional vs. ATP bioluminescence
- Test via ATP screen on day 6 and 14 to confirm method reliability
- Early time to detection demonstrated as valid
Performance variability

Build in forced changes

• Sample preparation step challenged, with handling of samples including vortex times, wiping tubing and time between sampling and aliquot

Verify at test site prior to execution of TT

• Training protocols provided
• Absence of product testing
• Confirmation exercise exercises
Review and Risk Assessment

Confirmatory studies at 3 inoculum levels with panel of 5 environmental isolates

Drug Product Method Suitability
Confirmatory study. Method suitability with compendial isolates. If limit product available, all rinse and nutrient media will be scaled down to reflect volume of drug product

Formulation Buffer: Method Suitability
Specificity studies with range of compendial and environmental isolates at target inoculum level of 10CFU. Performed on three batches of buffer in duplicate. Additional contamination from damaged bottles included

Product interference (Drug product)
Dilutions of product in nutrient media to confirm no interference with ATP assessment. Filtration of drug product with no rinse, following incubation confirmed no interference with ATP assessment

Performance variability: sample preparation, incubation time forced changes, slow growers

Media and sterility test canister variable suppliers

Equivalency Demonstrated; statistical evaluation. Includes compendial organisms, stressed, slow growers at range of inoculum levels

Include both options; traditional and rapid sterility in regulatory filings

Validation and tech transfer strategy

Tech Transfer to Sites; Primary Verification Study to confirm method operates as expected. Method suitability aligned to traditional sterility test

AstraZeneca

Vendor
Regulatory Engagement

- Strategically, AZ approach is to adopt during development phases and have early interactions with regulators on approach
- Scientific advice and briefing documents used
- Paper based exercise and some regulatory meetings to discuss further
- Platform approach taken to the MHRA innovation centre for discussion
- Shared our data and how we will “slot in” new products
- Dialogue with regulators open – feedback positive
- Clear benefit agreed for turbid products
Conclusions

• ATP Bioluminescence provides a reliable technique for microbial detection

• Clear understanding of the technology is required and it is important to ensure that users are provided with an overview of exactly what the technology is/isn’t doing

• Still hesitancy remains – change is hard!

• Our regulators expect us to utilise new technologies to enhance process robustness and understanding of our products
  • Shorter time to detection enables faster response to issues
Thank you.

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