Rapid Sterility Testing

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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 & Risk Assessment

- 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

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Testing Sites

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

AZ dev validation

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

AZ dev

Review and Risk Assessment

Vendor

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 demonstrate 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

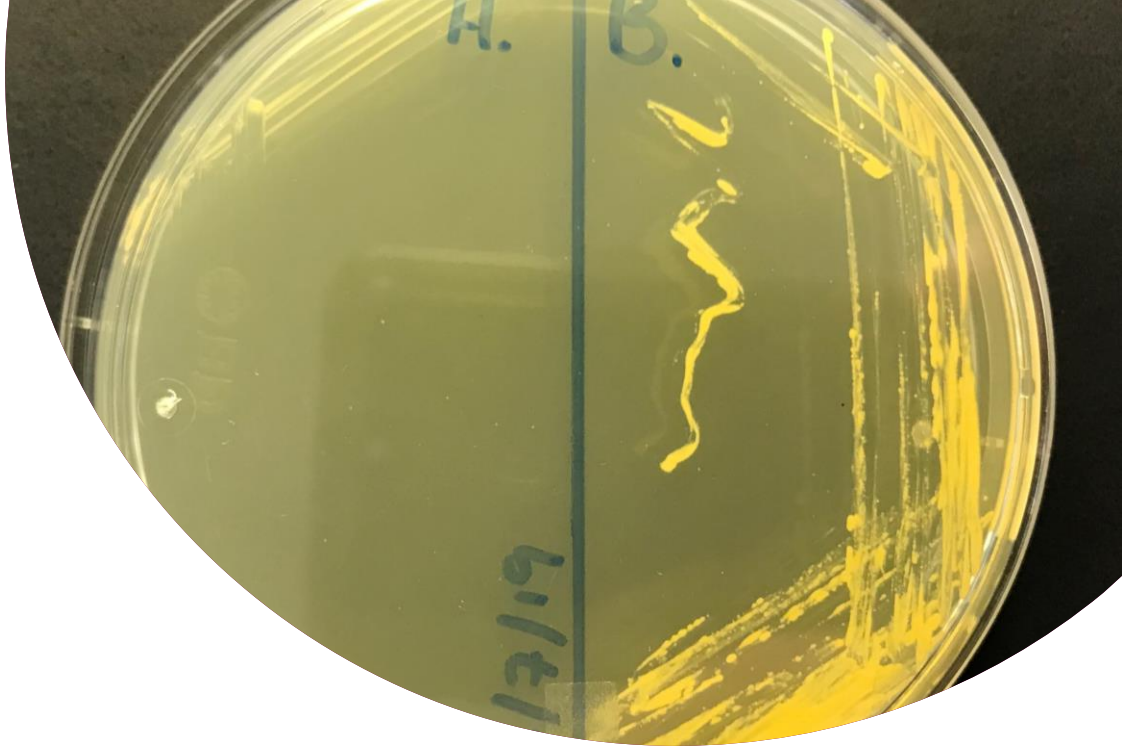
Environmental Isolates (AZ) - Specificity

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	Presence of Surrogate product	Alternative products
Three inoculum levels in absence of product; 10CFU, 1CFU, 0.1CFU *M. luteus, B. altitudinis, S. capitis*– using TSB & FTM *Penicillium chrysogenum* – using TSB *Cutibacterium acnes* - using FTM	On three lots of product, in duplicate; target 10CFU inoculum level *M. luteus, B. altitunas, S. capitis*– using TSB & FTM *Penicillium chrysogenum* – using TSB *Cutibacterium acnes* - using FTM	Risk assess but include this panel as a "standard"
Performed in duplicate as verification of specificity study		<10CFU target inoculum & following "AZ Panel" plus compendial *M. luteus,* *S. capitis, Cutibacterium acnes* – using FTM *Penicillium chrysogenum, B. altitudinis*– using TSB

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

Vendor Supplied

- Vendor supplied media used for equivalency study and majority of method validation.
- Mitigate risk of single supplier

Purchased media

- Purchased wide range of ready to use sterility testing media
- Background measured at different ages (including end of shelf life)
- Observed intra and inter-batch variability
- More background vs. Vendor supplied, however should a back-up be required Quality Control Checks Outlined

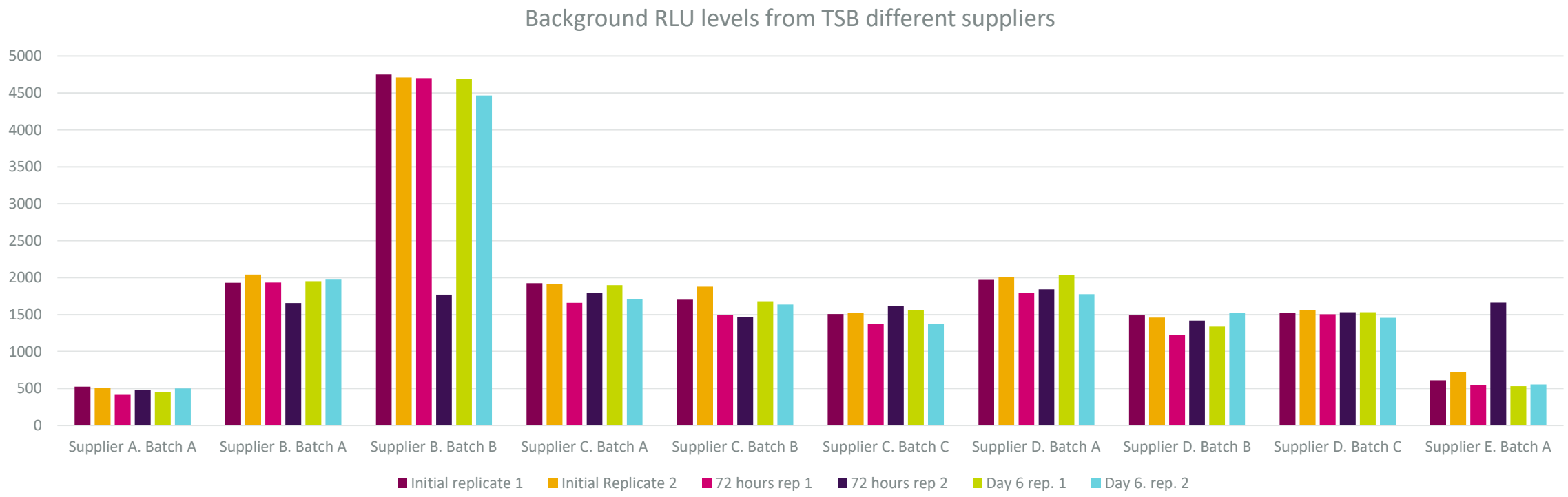
In-house preparation

- Greatest variability both within a batch of media and across batches
- Process improved with “new” vials vs. recycled
- Unlikely to use routinely

Rinse Fluids

- Generated data from canisters using range of rinse fluids, suppliers, types, batches, in–house prep
- No significant difference observed within the testing framework

Background monitoring of nutrient media, across suppliers



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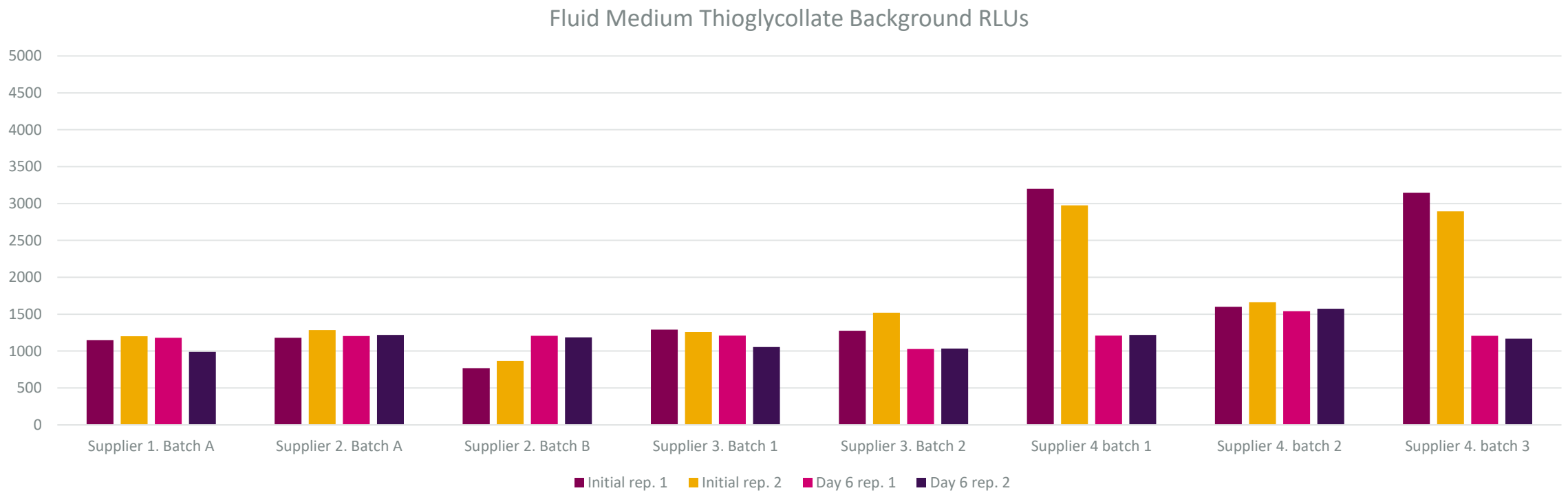
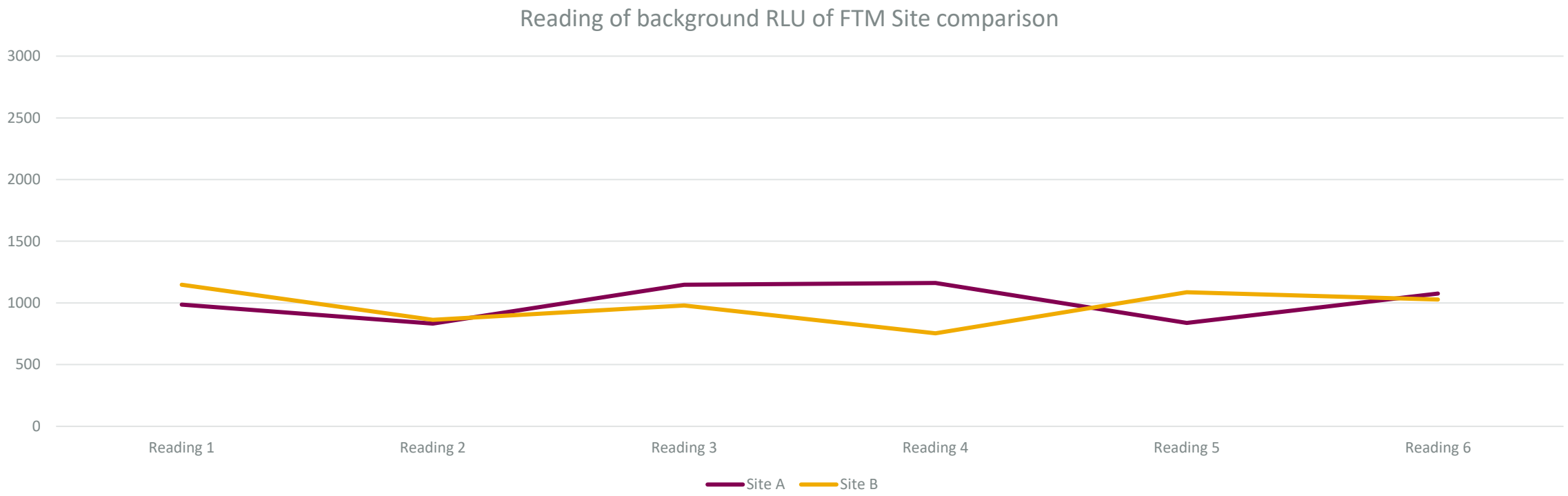


Within batch performance



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

Time to result

Organism	Inoculum count (cfu)	Time to Result Visual	Time to Result ATP
Aspergillus brasiliensis	2	Day 6	Day 6
Penicillium chyrsogenum	12	72 hours or Day 6	72 hours
C. acnes	5	Day 5-11	24 hours

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

Product	Background RLU
Product A	990
Product B	2346
Product C	1065402
Product D	863
Product E	873
Product F	686
Product G	78

Overcoming Sample Effects

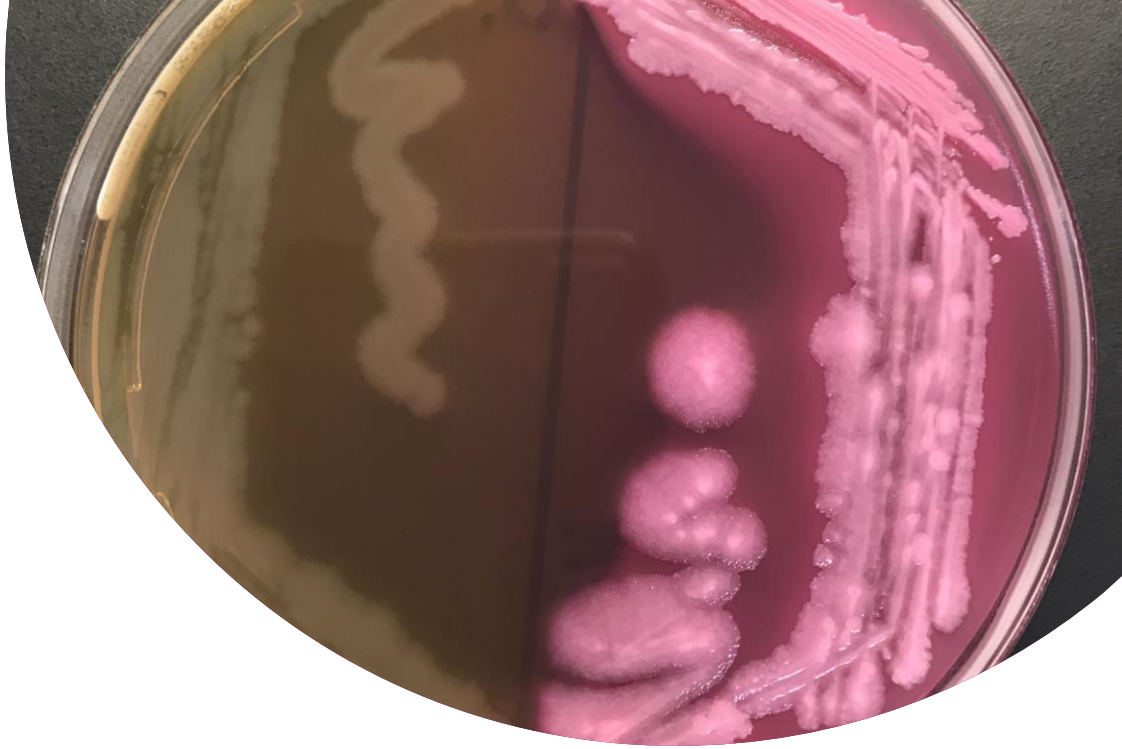
Sample effects

- 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”

Example of Filtration Data

Sample	Unfiltered 1	Cellulose filtered	Unfiltered 2	PVDF filtered
1:10 FTM dilution	NT	NT	131112	108412
1:100 FTM Dilution	28474	2971	7909	6297

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

Validation and tech transfer strategy

Release sites

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

Include both options; traditional and rapid sterility in regulatory filings

AstraZeneca

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 damage 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

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

Product specific

Review and Risk Assessment

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

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

Media and sterility test cannister variable suppliers

Abs of product

Vendor

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

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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