• Introduction
  – Background to Sterility test [ST]
  – Conventional test outline
  – Conventional test issues
  – Use of ST in cell & gene therapy manufacture processes

• Overview of Alternative Technologies:
  – BacT/Alert
  – Rapid Sterility Test Approach (RaSTA- IUK)

• Some Considerations

• Acknowledgements

• Questions
Introduction

Compendial sterility test outline & issues
Sterility Test

• Assessment of whether a sterilised medicinal product (inc intermediates/ bulks) is free from contaminating micro-organisms.
  – Represents only a small part of assurance….
  – Incubate whole or part of product with nutrient media and look for signs of growth/ turbidity.
  – Essential due diligence for patient safety & product quality

• The test first appeared in 1932 (British Pharmacopoeia).
  – Featured two media, method suitability and a defined incubation time (5 days!). Allowed two retests (all three had to fail to fail the test).
  – No test can actually test for sterility as sterility is an absolute term
  – The test is growth-based and requires microorganisms capable of active replication

• Since then incubation time has got longer –
  – much longer 14 days to include regulatory concerns around recovery of slow growing or compromised organisms
  – Technically some modifications seen e.g Self-contained sample filtration canisters – eg Steritest
  – But basic premise of test methodology remains the same
### NEED
- Challenge is for a rapid sterility test for Cell Therapy products to allow speedy intervention in the disease state.
- Current tests are designed for small molecules with a long shelf life.
- Currently, cell & gene therapy sterility testing done during manufacture process, and **products released at risk**.
- Radical transformation in methodology is needed to detect microbial contamination rapidly.

### IMPACT
- Patient safety
- Product quality
- Rapid sterility test results for cell & gene therapy products in hours compared to days
- Modality focused ST
- Move away from autologous cell & gene therapy at risk product release

### STATUS
- Mid-way thro' a 30 month IUK project to develop a PCR and digital PCR approach to a rapid sterility test (<1hr), with 2 tests with detection of viable microbial contaminants – moving towards a truly rapid sterility test.
Sterility Testing
Compendial Method and BacT/ALERT

RaSTA (Rapid Sterility Testing Approach) Project Overview

Compendial Method

- Sample cultured in various media for 14-days and visual results analysed by trained sterility professional
- Soybean casein digest media [Fungi and aerobic bacteria]
- Fluid thioglycolate media [Aerobic and anaerobic bacteria]
- Culture for 14 days
- Turbidity test to confirm presence of bacteria and fungi
- Test to confirm specific microbes

BacT/ALERT 3D Dual-T

- Capacity: 480 or greater; Incubation at 22.5 and 32.5°C.
Limitations of the compendial sterility test

- Not all microorganisms grow under test conditions (VBNC).
- Subjective end point – operator must be trained to detect growth (turbidity).
- Data integrity issues associated with subjectivity of the visual examination of the results.
- 14-day test not suitable for short life products like compounded sterile preparation and autologous cell therapy products.
- Turbidity end point not suitable for some products (opaque suspensions/solutions).

Sterility Test Time to result comparison

- HVLD
- Rasta
- BacTalert
- Comp MF ST
- Comp Direct ST

Time to result

• Growth based end point

* Compendial membrane filtration, ** Compendial direct inoculation
Rapid Method Development – Sterility Test

Compendial growth-based Sterility test - situation

- Labor-intensive
- Slow (can take up to 14-19 days for results).
- Real time/ Near real time results not possible
- In existence for many years (1932) with few changes
- Not suitable for some new or challenging drug formulations

Target

Develop new technology platforms for products where traditional tests are not applicable.

- **Rapid Sterility Test** (Product release/ In-process Control)
  BacT/Alert – applications for sterile small molecule, Cell & Gene Therapy, Biopharm
  Rasta – Cell & Gene Therapy
- **Stability Sterility Test Surrogate**
  High Voltage Leak Detection (HVLD): Dye immersion/microbial ingress cannot be used for certain products (i.e. suspensions) to assure container integrity (CCI - sterility indicator for stability testing).
Sterility Checks Associated with Autologous Cell & Gene Therapy Manufacture

Day 0

Bone Marrow Received

- Cell Count
- IPC Sterility
- Immunophenotype

Pre-stimulation

- Cell Count
- IPC Sterility
- Immunophenotype

Transduction 1

- Cell Count
- Clonogenic Capacity
- Immunophenotype
- IPC Sterility

Transduction 2

- Cell Count
- IPC Sterility
- Immunophenotype

Drug Product processing

- Cell Count
- Viability
- Sterility
- Mycoplasma
- Immunophenotype

Final Drug Product

Patient infusion

Product Used Prior to final ST results

Sterility check if cryopreservation used

Day 7
Alternative Sterility Test

BacT/Alert 3D dual temperature – Product & In Process Control
BacT/Alert

Overview

• Automated colorimetric technology
• Robust detection of bacteria, yeast and mold
• BacT/ALERT Industry Culture Media offer the ability to detect aerobic, anaerobic, and facultative anaerobic microorganisms, as well as yeast and fungi.
• The patented colorimetric sensor-and-detection technology detects microorganisms by tracking CO₂ production,
• Direct Inoculation
• Non-destructive sterility test method

Benefits

• Detection normally occurs within 24-72 hours.
• Continuous monitoring, bottles are read every 10 minutes
• User notification of positives is immediate and recorded (DI)
• Objective test results, no need to manually interpret turbid samples
• Reduction in hands-on time and less labor intensive (reduced contamination potential) eg. 10h v 64h estimate for Cabotegravir
• Higher testing throughput potential due to reduced incubator footprint & simpler sample prep (480 samples)
• Commercially available technology used in other sectors for 20 years.
Sterility Testing

*BacT/ALERT*

- Detects changes in pH resulting from gas emitted by microorganisms growing in culture media;
  - Viable microorganisms produce CO$_2$, this diffuses across the membrane into the sensor
  - In the presence of CO$_2$ the sensor turns from blue/dark green to yellow
  - This colour change is detected by a red-light-absorbing photodiode and alerts the user of the positive result.
  - Scans each bottle every 10 min
  - Runs for 7 days
  - Included in regulatory filing by GSK for cell & gene therapy application in 2016
  - Data Integrity compliant
Rapid Sterility Test

Rasta – Cell & Gene Therapy innovation project
An Innovate UK project in collaboration with Catapult and LGC
PCR based nucleic acid tests for rapid sterility
Potential benefits and challenges

Advantages

• Using degenerate primers to target 16s and 18s rDNA sequences that will cover potentially ~ 90% of bacterial and ~ 80% fungal species

• High sensitivity of PCR amplification methods (comparable to compendial tests)

• Detection of Viable Non Culturable organisms

• Potential to be very rapid (hours vs days)

• Use technologies that are readily available in most QC laboratories

Challenges

• Using degenerate primers to target 16s and 18s rDNA sequences that will potentially cover ~ 90% of bacterial and ~ 80% fungal species

• High sensitivity of PCR amplification methods (comparable to compendial tests)

• Detection of Viable Non Culturable organisms

• Detection of microbial target DNA in background of ~ 20 million human cells per ml (needle in a haystack!)

• Interference from contaminating sources of DNA (non-viable organisms, reagents, raw materials)

• Comparison of genome detection with Colony Forming Unit (CFU)
**Rasta Technologies**
UK Government sponsored project – Innovate UK - collaboration between GSK, Catapult & LGC

**LGC** – at the forefront of DNA probe forensic technology.

- Develop rapid (< 1hr.), sensitive PCR/RT-PCR based sterility test for microbial detection.
- LGC’s ParaDNA amplification technology uses fluorescent HyBeacon™ probe detection of PCR amplicons.
- Primers to 16s ribosome for bacteria and 18s for fungi.

**Cell Therapy Catapult (CTC)- UK Government sector**

- CTC mission is to accelerate the development of cell and gene therapies and associated technologies in the UK.
- Develop ultrasensitive digital PCR assay for viable microbial detection.
- Assay will run 20,000 simultaneous PCR reactions from a single sample with aim for single copy number sensitivity.
- Droplet digital PCR platform (Bio-Rad).
- Sample preparation protocol development.
Combining 2 new platforms to test sterility in ~1 hour

- ParaDNA from LGC - QUALITATIVE
- Digital PCR from Cell Therapy Catapult - QUALITATIVE

Comparability studies (underway) of new platforms against BacT/ALERT

Platform validation will follow

**Aerobic medium**
- Staphylococcus aureus
- Bacillus subtilis
- Pseudomonas aeruginosa
- Candida albicans
- Aspergillus brasiliensis

**Anaerobic medium**
- Clostridium sporogenes
- Bacteroides fragilis

Suggested comparability design

EP 2.6.27. Microbiological examination of cell-based preparations
ParaDNA Platform
LGC, Cambridge/London

RaSTA (Rapid Sterility Testing Approach) Project Overview

- Rapid detection (~1 hour) of a huge array of bacteria and fungi via PCR
- Simple kit testing method
- Utilises 16s and 18s primers
- IPCs in each well
- Reports DETECTED or NOT DETECTED for contamination
Benefits

• **Speed to result** – real time/near real time result generation

• **Environmental**: Reduced power consumption for incubators, autoclave use, plastics use and media use and disposal.

• **Antibiotic usage**: Reduction in treatment with prophylactic antibiotics due to microbiological contaminated product.

• **Improved risk profile**: increased confidence through improved risk management and greater process characterisation (troubleshooting and in process control).

• **Future markets**: The test may also have applications in other market sectors such as biopharmaceuticals (Cell lines), clinical, the food and cosmetic industry where 42% of recalls of products in the US are related to microbial contamination.

• Simple & cost effective (10 fold reduction vs compendial test)
Some final thoughts
Advocacy, Progress and Potential Applications
Formulation considerations

• Fresh cell & gene therapy products: patient safety and need for rapid disease state intervention means rapid sterility testing is essential

• Frozen cell & gene therapy products: speed of test
  – Major impact on microbiological process control & product quality
Alternative methods – Regulatory Advocacy

• Demonstration of performance equivalence using the validation parameters

• Validation Strategy
  ➢ Pharm Eur 2.6.27 Microbiological examination of cell-based preparations
  ➢ USP <1223> Validation of Alternative Microbiological Testing Methods/
  ➢ Ph. Eur 5.1.6. Alternative methods for control of microbiological quality.
  ➢ PDA Technical report 33 – Evaluation, validation and implementation of alternative and rapid microbiological methods.

• Regulatory Support for Alternative Methods
  ➢ The current FDA strategic plan acknowledges that analytical technologies are rapidly changing and leading to dramatic improvements in sensitivity, resolution, and precision in the detection of contaminants.
  ➢ Ph. Eur 5.1.6 - aims to facilitate the implementation and use of alternative microbiological methods where this can lead to efficient microbiological control and improved assurance for the quality of pharmaceutical products.
  ➢ MHRA – Innovation group – set up to consider & advise on implementation & use of alternative technologies
RMM issues from a MHRA perspective

Andrew Hopkins, MHRA senior inspector – Pharmig conference Nov 2017

• RMM already in use (but needs more uptake)
• Regulators are keen to support innovation
• Help the regulator to understand
• Caution
• Be careful what you ignore
• Still need the right knowledge
• Additional tool in the tool box does not necessarily replace existing tools

• Talk to Regulators!