Suitability of Microbial Limits Test Methods

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MicroWorks, Inc.
MicroWorks, Inc.

- cGMP compliant Microbiological test laboratory located in Crown Point, IN
- Testing includes sterility, endotoxin analysis, raw material testing, Microbial Limits testing, Container closure integrity testing
- DEA licensed, Schedule 2-5
- Microbiological consulting services including qualification of cleanrooms (EMPQ), Set up of EM Programs, Micro method validation
Course Objectives

- High level understanding of the Microbial limits test
- References
- Setting up the test
- Transfer Steps
- Reading the Results
- Suitability testing
- Release of Media
Microbial Limits

- Testing performed on non-sterile products to demonstrate they are suitable for their intended use.
- Quantitative testing- Result in a number indicating the amount of bioburden detected in the product.
- Qualitative-Results in a positive or negative result.
References

- **USP 61**
  - Quantitative testing
  - Enumeration of mesophilic bacteria and fungi that grow under aerobic conditions.
  - Can be performed by pour plating, spread plating or membrane filtration.
  - Most probable number procedures may be used where levels are very low.

- **USP 62**
  - Qualitative testing
  - Also known as testing for specified organisms.
  - Test included in the chapter include: *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Bile-Tolerant Gram Negative (BTGN), *Clostridium*, and *Candida albicans*. 
USP <1111>

- Informational chapter that goes with USP <61> and <62>
- Provides guidance regarding acceptance criteria
- The route of administration plays a key role in determining what is an acceptable limit.
- The health of the intended user plays a role as well.
Setting up the <61> Test

- The amount of product to be tested is weighed or pipetted into a diluent (may be phosphate buffer, TSB, letheen broth, DE broth, or other suitable diluent) to make a 1:10 dilution.
- Typically 10g is tested.
- The pH of the diluent is adjusted so that it is in the 6-8 range.
- The product is dissolved by shaking or vortexing until it goes into solution.
Setting up the <61> Test

- The sample is then pipetted into plates and media is added.
- For TAMC (Total aerobic microbial count) the media is typically TSA or TSA with lecithin and tween. Incubation is 30-35°C for 3-5 days.
- For TYMC (Total yeast and mold count) the media is typically SDA or SDA with lecithin and tween. Incubation is 20-25°C for 5-7 days.
Plating samples for USP <61>

- TSA or TSA with lecithin and tween is added to two of the plates. These plates are incubated at 30-35C for 3-5 days.
- SDA or SDA with lecithin and tween is added to two of the plates. These plates are incubated at 20-15C for 5-7 days.
- Different media may be needed depending on method development/validation.
Setting up USP <61>

- 10g of product is dissolved in diluent
- may be TSB, Lactose broth, phosphate buffer, D/E Neutralizer or other diluent that is shown to be suitable.
- The pH of the solution is adjusted to be between 6-8.
- 1 mL aliquots are aseptically plated.
Reading USP <61> Plates

- Once plate incubation is completed the plates are examined for colonies.
- The number of colonies are counted and the results are calculated, taking into consideration what dilution was plated.
- For example: If the colony counts are 6 on one plate and 10 on the other. The total is 16/2 for an average count of 8. If the test was a 1/10 dilution the result is multiplied by 10 so the result is 80 cfu/g.
Setting up the <62> Testing

- Depending on the organisms that are specified the method will vary.
- Organisms for testing based on specification of the product, compendial specification, route of administration, country where it will be sold, origin of sample, etc.
- The amount of sample to be tested is typically 1-10 g. Testing for *E.coli* and *Salmonella* are typically 10g.
USP <62> Testing

Attachment 2. USP <62> Testing Flowcharts
BTGN, E. coli, Salmonella

**Bile Tolerant Gram Negative**
- 1 mL or g into 9mL TSB/LB
  - Inc. 2-5 hrs @ 20-25°C
  - Transfer entire 10mL to 90mL EBM
  - Inc. 24-48 hrs @ 30-35°C
  - Subculture to VRBG agar
  - Inc. NLT 22 hrs @ 30-35°C
  - Read plates, interpret results

**Escherichia coli**
- 1mL or g into 99mL TSB/LB
  - Inc. 22-24 hrs @ 30-35°C
  - Transfer 1mL to 100mL MCB
  - Inc. 24-48 hrs @ 42-44°C
  - Subculture to MCA
  - Inc. NLT 22 hrs @ 30-35°C
  - Read plates, interpret results

**Salmonella**
- 10mL or g into 90mL TSB/LB
  - Inc. 22-24 hrs @ 30-35°C
  - Transfer 0.1mL to 10mL RVS broth
  - Inc. 22-24 hrs @ 30-35°C
  - Subculture to XLD
  - Inc. NLT 22 hrs @ 30-35°C
  - Read plates, interpret results
Pseudomonas aeruginosa
1mL or g into 99mL TSB/LB
Inc. 22-24 hrs @ 30-35°C
Subculture to CMA
Inc. NLT 22 hrs @ 30-35°C
Read plates, interpret results

Staphylococcus aureus
1mL or g into 99mL TSB/LB
Inc. 22-24 hrs @ 30-35°C
Subculture to MSA
Inc. NLT 22 hrs @ 30-35°C
Read plates, interpret results

Candida albicans
1mL or g into 9mL SDB/TSB/LB
Transfer 10mL to 100mL SDB
Subculture to CMA
Inc. 3-5 days @ 30-35°C
Subculture to SDA
Inc. NLT 24 hrs @ 30-35°C
Read plates, interpret results

Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans
USP <62> Testing

Attachment 4. USP <62> Testing Flowchart
Clostridia

2 containers: 1mL or g into 9mL TSB/LB

Heat shock 1 container for 10 min @ 80±2°C. Cool rapidly. Do not heat the other containers

Transfer 10mL to 90mL RMC (2 separate containers)

Inc. 48 hrs @ 30-35°C anaerobically

Subculture to CLA

Inc. NLT 48 hrs @ 30-35°C anaerobically

Read plates, interpret results

Inc. 48 hrs @ 30-35°C anaerobically
Since this is a qualitative test record whether or not there is growth on the plate.

If there is growth on the plate determine if the growth is the organism being tested for.

Gram stain the organism and observe it microscopically.

If the organism is not the same morphology and gram reaction as the organism being tested the test is negative for the target organism.
Reading Results for USP <62>

- If the organism appears to be the same morphology and gram reaction as the organism being tested for perform an ID of the organism for confirmation.
- If the organism is the same as the organism being tested for the results is positive.
- If the organism is not the same as the organism being tested for it is reported as negative.
- Need to investigate the impact of the organism.
Method Suitability

- Method suitability is performed for each product that is to be tested by the Microbial Limits method.
- This is done to ensure that any inhibition that the product may have is overcome by the test method to ensure if contamination is present in the sample it will be detected.
Method Suitability for <61>

- To demonstrate that a variety of organisms can be detected in the presence of the sample.
- Sample is prepared according to the proposed method.
- pH is adjusted to between 6-8.
- Aliquots of the sample are spiked with representative organisms.
- Organisms plated without the presence of the product. These plates are the positive controls.
Method Suitability for USP <61>

- Representative Organisms include:
  - *Staphylococcus aureus* (SA)-gram positive cocci
  - *Pseudomonas aeruginosa* (PA)-gram negative rods
  - *Bacillus subtilis* (BS)-gram positive rods with spores
  - *Aspergillus brasiliensis* (AB)-mold
  - *Candida albicans* (CA)-yeast
Method Suitability for USP <61>

- Bacteria are plated with TSA or TSA with lecithin and tween and incubated at 30-35°C for ≤3 days.
- Yeast and mold are plated on TSA or TSA with lecithin and tween and incubated at 30-35°C for ≤5 days and on SDA or SDA with lecithin and tween and incubated at 20-25°C for ≤5 days.
- Other media may need to be substituted based on method development.
Method Suitability for USP <61>

- After incubation the plates are counted and the plates with the product (test samples) are compared to the positive control plates to see if the product caused inhibition.

- The percent recovery is calculated:
  \[
  \text{Percent recovery} = \frac{\text{Test Sample}}{\text{Positive Control}} \times 100
  \]
Method Suitability for USP <61>

- Percent recovery must be between 50 and 200% for all organisms for the test to be valid.
- If any of the organisms are inhibited, modifications of the method are needed to overcome the inhibition.
- This might include dilution, using a different primary enrichment, using a different media to plate, extending the incubation, filtering the product....
## Example of Suitable TAMC Method

<table>
<thead>
<tr>
<th>Organism Challenge</th>
<th>Inoculum Count (cfu/plate)</th>
<th>% Recovery</th>
<th>Meets Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>34.5</td>
<td>113%</td>
<td>Yes</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>36.5</td>
<td>99%</td>
<td>Yes</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>42.5</td>
<td>94%</td>
<td>Yes</td>
</tr>
<tr>
<td>Aspergillus brasiliensis</td>
<td>33.5</td>
<td>78%</td>
<td>Yes</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>32.5</td>
<td>105%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Summary of sample preparation and testing:

<table>
<thead>
<tr>
<th>Method Suitability</th>
<th>Routine Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weighed 10g product into a sterile container. Prepared a 1:10 dilution by adding</td>
<td>Weigh a minimum of 10g product into a sterile container. Add Phosphate Buffer to</td>
</tr>
<tr>
<td>enough Phosphate Buffer to equal 100g. Adjusted pH by adding 0.4 mL 2M NaOH.</td>
<td>prepare a 1:10 dilution. Adjust pH by adding 0.4 mL 2M NaOH per 100 mL prepared</td>
</tr>
<tr>
<td></td>
<td>sample solution.</td>
</tr>
<tr>
<td>Pour-plated 1mL in duplicate with TSA.</td>
<td>Pour-plate 1mL in duplicate with TSA.</td>
</tr>
<tr>
<td>Incubated for 44 hours and 20 minutes at 30-35° C.</td>
<td>Incubate for 3–5 days at 30-35° C.</td>
</tr>
</tbody>
</table>
**Example of Suitable TYMC Method**

<table>
<thead>
<tr>
<th>Organism Challenge</th>
<th>Inoculum Count</th>
<th>% Recovery</th>
<th>Meets Acceptance Criteria Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus brasiliensis</td>
<td>31.5</td>
<td>92%</td>
<td>Yes</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>43.5</td>
<td>123%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Summary of sample preparation and testing:**

<table>
<thead>
<tr>
<th>Method Suitability</th>
<th>Routine Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weighed 10g product into a sterile container. Prepared a 1:10 dilution by adding enough Phosphate Buffer to equal 100g. Adjusted pH by adding 0.4 mL 2M NaOH.</td>
<td>Weigh a minimum of 10g product into a sterile container. Add Phosphate Buffer to prepare a 1:10 dilution. Adjust pH by adding 0.4 mL 2M NaOH per 100 mL prepared sample solution.</td>
</tr>
<tr>
<td>Pour-plated 1mL in duplicate with SDA.</td>
<td>Pour-plate 1mL in duplicate with SDA.</td>
</tr>
<tr>
<td>Incubated for 49 hours and 20 minutes at 20-25°C.</td>
<td>Incubate for 5–7 days at 20-25°C.</td>
</tr>
</tbody>
</table>
Suitability for USP 62

- Each specified organism requires a separate suitability test.
- Incubation steps are all performed at <time that the step will be performed routinely.
- For example, if method states, incubate for 18-24 hours, the suitability testing needs to be performed at <18 hours.
<table>
<thead>
<tr>
<th>Day</th>
<th>BTGN</th>
<th>EC</th>
<th>SE</th>
<th>PA</th>
<th>SA</th>
<th>CS</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>1g/1mL Product into 9mL TSB/LB</td>
<td>1g/1mL Product into 99mL TSB/LB</td>
<td>10g/10mL Product into 90mL TSB/LB</td>
<td>1g/1mL Product into 99mL TSB/LB</td>
<td>1g/1mL Product into 99mL TSB/LB</td>
<td>Heat one tube for 10 min. at 80°C</td>
<td>1g/1mL Product into 9mL SDB/TSB/LB</td>
</tr>
<tr>
<td></td>
<td>Inc. ≤2 h at 20-25°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Transfer contents of each to 100mL RMC</td>
<td>Transfer 10mL to 100mL SDB</td>
</tr>
<tr>
<td></td>
<td>Transfer each 10mL TSB/LB to 90mL EBM broth</td>
<td>Inc. ≤24 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤48 h at 30-35°C</td>
<td>Inc. ≤3 days at 30-35°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subculture to VRBG agar</td>
<td>Subculture to CMA</td>
<td>Subculture to MSA</td>
<td>Subculture to CMA</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td>Transfer 1mL to 100mL MCB</td>
<td>Inc. ≤22 h at 42-44°C</td>
<td>Inc. ≤24 h at 30-35°C</td>
<td>Inc. ≤48 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transfer 0.1mL to 10mL RVS broth</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subculture to XLD</td>
<td>Subculture to XLD</td>
<td>Subculture to CLA</td>
<td>N/A</td>
<td>Subculture to SDA</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>Read plates</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤24 h at 30-35°C C, anaerobic</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subculture to MCA</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Inc. ≤22 h at 30-35°C</td>
<td>Subculture to CLA</td>
<td>Subculture to SDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subculture to XLD</td>
<td>Read plates</td>
<td>Read plates</td>
<td>Inc. ≤24 h at 30-35°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>Read plates</td>
<td>Read plates</td>
<td>Read plates</td>
<td>N/A</td>
<td>Inc. ≤24 h at 30-35°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Example USP <62> Suitability Flowcharts

**Bile Tolerant Gram Negative (BTGN)**
- Test EC: 1mL/1g into 9mL TSB/LB
  - Pos Ctrl EC: 10mL TSB/LB
  - Test PA: 1mL/1g into 9mL TSB/LB
  - Pos Ctrl PA: 10mL TSB/LB
  - Inoculate each with ≤100cfu EC or PA
- Inc. ≤ 2 hrs
  @ 20-25°C
- Transfer entire 10mL to 90mL EBM
- Inc. ≤ 24 hrs
  @ 30-35°C
- Subculture to VLBG
- Inc. ≤ 22 hrs
  @ 30-35°C
- Read plates, interpret results

**Escherichia coli**
- Test EC: 1mL/1g into 99mL TSB/LB
  - Pos Ctrl EC: 100 mL TSB/LB
  - Inoculate each with ≤100cfu EC
- Inc. ≤ 22 hrs
  @ 30-35°C
- Transfer 1mL to 100mL MCB
- Inc. ≤ 24 hrs
  @ 42-44°C
- Subculture to MCA
- Inc. ≤ 22 hrs
  @ 30-35°C
- Read plates, interpret results

**Salmonella**
- Test SE: 10mL/10g into 90mL TSB/LB
  - Pos Ctrl SE: 100 mL TSB/LB
  - Inoculate each with ≤100cfu SE
- Inc. ≤ 22 hrs
  @ 30-35°C
- Transfer 0.1mL to 10mL RVS broth
- Inc. ≤ 22 hrs
  @ 30-35°C
- Subculture to XLD
- Inc. ≤ 22 hrs
  @ 30-35°C
- Read plates, interpret results
**Pseudomonas aeruginosa**

Test PA: 1mL/1g into 99mL TSB/LB  
Pos Ctrl PA: 100mL TSB/LB  
Inoculate each with ≤100cfu PA

Inc. ≤ 22 hrs  
@ 30-35° C

Inc. ≤ 22 hrs  
@ 30-35° C

Read plates, interpret results

**Staphylococcus aureus**

Test SA: 1mL/1g into 99mL TSB/LB  
Pos Ctrl: 100 mL TSB/LB  
Inoculate each with ≤100cfu SA

Inc. ≤ 22 hrs  
@ 30-35° C

Subculture to MSA

Inc. ≤ 22 hrs  
@ 30-35° C

Read plates, interpret results

**Candida albicans**

Test CA: 1mL/1g into 9mL SDB/TSB/LB  
Pos Ctrl CA: 10mL SDB/TSB/LB  
Inoculate each with ≤100cfu CA

Inc. ≤ 3 days  
@ 30-35° C

Transfer entire 10mL to 100mL SDB

Inc. ≤ 22 hrs  
@ 30-35° C

Subculture to SDA

Inc. ≤ 24 hrs  
@ 30-35° C

Read plates, interpret results
2 Test CS: 1mL/1g into 9mL TSB/LB
2 Pos Ctrl CS: 10 mL TSB/LB
Inoculate each with ≤100cfu CS

Heat shock 1 Test and 1 Pos Ctrl for 10 min @ 80±2°C. Cool rapidly. Do not heat the other two containers

Transfer 10mL to 90mL RMC (4 separate containers)

Inc. ≤ 48 hrs @ 30-35°C anaerobically

Subculture to CLA

Inc. ≤ 48 hrs @ 30-35°C anaerobically

Read plates, interpret results
Acceptance Criteria for USP <62> Suitability

- Inoculum of challenge organism must be <100 cfu.
- Negative control must be negative.
- Growth obtained from challenge test must be comparable to growth of positive control. If not, additional method development work is needed.
Media for Microbial Limits Testing

- Media for USP <62> testing is specifically to select for the target organism and to inhibit other organisms that might compete with the target organism.

- Releasing selective media: Inoculate with <100 cfu of the target organism. Incubate at less than the shortest time it will be incubated.

- Also inoculate with >100 cfu of organisms that should be inhibited.
Setting up Media Release
<table>
<thead>
<tr>
<th>Media</th>
<th>Organism</th>
<th>ATCC No.</th>
<th>Inoculum CFU</th>
<th>Incubation Temp</th>
<th>Incubation Time</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSA</td>
<td>S. aureus</td>
<td>6538</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 3 days</td>
<td>Round yellow colonies</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>9027</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 3 days</td>
<td>Yellow-green to blue-green undulate round colonies</td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
<td>6633</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 3 days</td>
<td>Large flat colonies</td>
</tr>
<tr>
<td></td>
<td>A. brasiliensis</td>
<td>16404</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 5 days</td>
<td>Fuzzy black colonies</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>10231</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 5 days</td>
<td>Large milky colonies</td>
</tr>
<tr>
<td>(RMC)</td>
<td>C. sporogenes</td>
<td>11437 or 19404</td>
<td>≤100</td>
<td>30-35°C anaerobic</td>
<td>≤ 48 hrs</td>
<td>Growth</td>
</tr>
<tr>
<td>Reinforced Medium for Clostridia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LB)</td>
<td>P. aeruginosa</td>
<td>9027</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 48 hrs</td>
<td>Growth</td>
</tr>
<tr>
<td>Letheen Broth</td>
<td>B. subtilis</td>
<td>6633</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 48 hrs</td>
<td>Growth</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>6538</td>
<td>≤100</td>
<td>30-35°C</td>
<td>≤ 48 hrs</td>
<td>Growth</td>
</tr>
</tbody>
</table>
## Growth Promotion Testing for Selective Media

<table>
<thead>
<tr>
<th>Media</th>
<th>Organism</th>
<th>ATCC No., or equivalent</th>
<th>Inoculum CFU</th>
<th>Incubation Temp</th>
<th>Incubation Time</th>
<th>Expected Results</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CMA) Cetrimide Agar</td>
<td><em>P. aeruginosa</em></td>
<td>9027</td>
<td>≤100</td>
<td>30-35° C</td>
<td>≤ 22 hrs</td>
<td>Growth promoting</td>
<td>Yellow green to blue colonies</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>8739</td>
<td>≥100</td>
<td>30-35° C</td>
<td>≥ 72 hrs</td>
<td>Inhibitory</td>
<td>Inhibition of growth</td>
</tr>
<tr>
<td>(M-Endo) M-Endo Agar</td>
<td><em>P. aeruginosa</em></td>
<td>9027</td>
<td>≤100</td>
<td>30-35° C</td>
<td>≤ 48 hrs</td>
<td>Atypical</td>
<td>Clear to opaque colonies; no red color</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>8739</td>
<td>≤100</td>
<td>30-35° C</td>
<td>≤ 48 hrs</td>
<td>Typical</td>
<td>Red colonies with greenish metallic sheen</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>6538</td>
<td>≥100</td>
<td>30-35° C</td>
<td>≥ 48 hrs</td>
<td>-</td>
<td>Marked to complete inhibition</td>
</tr>
<tr>
<td>(MSA) Mannitol Salt Agar</td>
<td><em>S. aureus</em></td>
<td>6538</td>
<td>≤ 100</td>
<td>30-35° C</td>
<td>≤ 22 hrs</td>
<td>Growth promoting + indicative</td>
<td>Small to large colonies with yellow zones</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>8739</td>
<td>≥ 100</td>
<td>30-35° C</td>
<td>≥ 72 hrs</td>
<td>Inhibitory</td>
<td>Marked to complete inhibition</td>
</tr>
</tbody>
</table>
Summary

- Microbial Limits testing is one of the most common test methods in the lab.
- All methods are shown to be suitable before tests are performed.
- The method that is used for suitability testing details how the sample is prepared and how long it is incubated.
- The setup of the test needs to follow the same method every time it is performed.
- All media needs specifications for release testing.
- Specifications vary from client to client and product to product so it is important to verify the requirements for each test.