USP Open Forum

Comparing Probiotic Plate Count Methods

June 16, 2022, 10:00 am - 12:30 pm ET
Virtual Meeting
The Analytical Procedure
Life Cycle Management for CFU Enumeration of Probiotics

Jean L. Schoeni, Ph.D.
Vice-Chair, Probiotic Expert Panel, and a Member of the Non-Botanical Dietary Supplements Expert Committee
Analytical Procedure Life Cycle Management (APLM)

FRAMEWORK

- APLM documents are guidelines
  - APLM is not enforcible
- You decide which APLM components fit your needs
- APLM can be implemented incrementally
- You can use historical data where applicable
GUIDELINES AND PUBLICATION

- Descriptions that include examples and tools are available
  - USP GC <1220>
    - Applies to all analytical procedures
  - “Improving and Comparing” article
    - Details application of APLM to probiotic plate count methods
  - Both are consistent with Quality by Design concepts described in International Council for Harmonisation guidelines
    - ICH Q14

(1220) ANALYTICAL PROCEDURE LIFE CYCLE

Frontiers in Microbiology

Improving and Comparing Probiotic Plate Count Methods by Analytical Procedure Lifecycle Management

M. L. Jane Weitzel, Christina S. Vegge, Marco Pane, Virginia S. Goldman, Binu Koshy, Cisse Hedegaard Porsby, Pierre Burguierre and Jean L. Schoeni

© 2021 USP
**BENEFITS**

- Practical and data driven approach to show your analytical procedure(s) and the results (reportable values) generated are fit for intended use.
- It makes the data that you use for making decisions more reliable.
BENEFITS

- Using high quality data helps improves the quality of probiotic products
  - Manufacturers will see greater product consistency and fewer OOS
  - Data can be systematically reviewed to evaluate processes
    - Driving innovations and efficiencies
    - Cost saving reductions in overage
  - Addresses the challenges of plate count methods
    - Greater assurance that beneficial doses will be delivered
What is APLM?

- **Analytical Procedure Life Cycle Management**

  A holistic approach that streamlines the management of analytical procedures (methods) throughout their life cycle
  - From design or selection of procedure, through modifications and validation, until retirement
What is APLM?

- **Analytical Procedure Life Cycle Management**
  
  - A holistic approach that streamlines the management of analytical procedures (methods) throughout their life cycle
    - From design or selection of procedure, through modifications, until retirement
  
  - A continuously developing knowledge base that becomes the cornerstone of communication for all discussions regarding a procedure and the products it supports
What is APLM?

- **Analytical Procedure Life Cycle Management**

  - A holistic approach that streamlines the management of analytical procedures (methods) throughout their life cycle
    - From design or selection of procedure, through modifications and validation, until retirement

  - A continuously developing knowledge base that becomes the cornerstone of communication for all discussions regarding a procedure and the products it supports

  - A qualification management system that ensures an analytical procedure remains fit for intended purpose throughout its use
How does APLM work?

Initial Information Gathering

- Measurand
  - Unambiguous description of what is being measured

- Decision Rule
  - Defines fitness requirements
  - Prescribes when to accept or reject a probiotic product

Analytical Target Profile (ATP)

- Predefined objective stating the performance requirements
  - Stipulates the quality of the reportable value (result)
  - Fit for intended use criteria

Three Stages

- 1: Procedure Design
- 2: Analytical Procedure Performance Qualification (APPQ)
- 3: Ongoing Procedure Performance Verification (OPPV)
What is the analyte? What is being counted?

What is the matrix? Are there excipients or stabilizers?

What is the physical form?

Are there possible contaminants in the matrix?

What are the units for the quantity?

Example for a *Lactobacillus* spp. ingredient

- Culturable cells (live cells freeze-dried) of *Lactobacillus* spp., CFU/g, in powder with cryoprotectant.
Decision Rule

- Description
- Decision unit
- Specification(s)
- Defined reportable result
- Standard uncertainty associated with the reportable value
- Acceptable probability for making an incorrect decision

Example for a *Lactobacillus* spp. ingredient

- The laboratory sample, taken from the batch of *Lactobacillus* spp. probiotic powder (culturable cells, freeze-dried) will be considered compliant with the specification of 10.962 Log$_{10}$ CFU/g if the reportable value is ≥10.962 Log$_{10}$ CFU/g, the MU is < 0.305 Log$_{10}$ CFU/g, and the probability of being wrong is ≤5%. Otherwise, it will be considered non-compliant.
How does APLM work?

Analytical Target Profile (ATP)

The Centerpiece of APLM

- The procedure must be able to enumerate the *Lactobacillus* spp. culturable cell count in CFU/g of powder with cryoprotectant, formulated to $11.462 \log_{10} \text{CFU/g}$, so the reportable values fall below a $TMU = 0.305 \log_{10} \text{CFU/g}$ (i.e., the $TMU$ associated with the reportable value is $< 0.305$) and the probability of being wrong is $\leq 5\%$. The plating range used by the laboratory will cover $9.462-13.462 \log_{10} \text{CFU/g}$, two $\log_{10}$ above and below the internal release specification.
How does APLM work?

Stage 1: Procedure Design

- Determine conditions that will help you achieve the accuracy and precision you want out of the procedure
  - Knowledge gathering
  - Experimentation
  - Risk assessment
  - Risk mitigation / Analytical Control Strategies
How does APLM work?

<table>
<thead>
<tr>
<th>Analytical Unit Operation</th>
<th>Analytical Factor or Variable</th>
<th>Identified Potential Risk</th>
<th>RISK HEAT MAP</th>
<th>Analytical Control Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAMPLE &amp; REAGENT PREPARATION</strong></td>
<td>Humidity of the laboratory</td>
<td>Moisture absorption by the sample can lead to incorrect weighing or degradation</td>
<td>Green</td>
<td>Monitor environmental controls</td>
</tr>
<tr>
<td></td>
<td>Analyst skill</td>
<td>Incorrect sample preparation; weighing &amp; volumetric dilutions</td>
<td>Red</td>
<td>Training program and records</td>
</tr>
<tr>
<td></td>
<td>Sonication time</td>
<td>Lack of dissolution of the sample or degradation</td>
<td>Green</td>
<td>Establish limit or conditions during development</td>
</tr>
<tr>
<td></td>
<td>Composition of the solvent mixture used in sample preparation</td>
<td>Lack of complete dissolution of the sample</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td><strong>INSTRUMENT &amp; SYSTEM SET UP</strong></td>
<td>% composition of the solvent in the mobile phase</td>
<td>Column performance, peak shape &amp; retention times</td>
<td>Red</td>
<td>Gravimetric preparation, SSTs</td>
</tr>
<tr>
<td></td>
<td>Column temperature</td>
<td></td>
<td></td>
<td>Establish operation within limits during instrument/system qualification; SSTs to confirm performance</td>
</tr>
<tr>
<td></td>
<td>Batch of column packing material</td>
<td></td>
<td></td>
<td>Establish variability during Stage 1 and design SSTs</td>
</tr>
<tr>
<td></td>
<td>Quality of the solvent</td>
<td>Baseline drift and noise are wavelength dependent and may affect the peak shape</td>
<td></td>
<td>Specify required grade and transmittance characteristics</td>
</tr>
<tr>
<td></td>
<td>Cleaning</td>
<td>Peaks from previous Injections</td>
<td></td>
<td>Establish cleaning protocol, SST</td>
</tr>
</tbody>
</table>

Risk

Assessment

USP GC <1220>
How does APLM work?

Stage 2: Analytical Procedure Performance Qualification (APPQ)

- Demonstrating “fit for intended use”
  - May include traditional validation, verification, or transfer activities
  - ANOVA experiments to allow calculation of uncertainties
How does APLM work?

<table>
<thead>
<tr>
<th>UNCERTAINTY COMPONENT</th>
<th>CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Analyst</td>
<td>A B A C</td>
</tr>
<tr>
<td>Lot of Plating Medium</td>
<td>1 2 1 2</td>
</tr>
<tr>
<td>Lot ofSuspension / Rehydration Medium</td>
<td>2 2 1 1</td>
</tr>
<tr>
<td>Lot of Dilution Buffer</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Disposable Serological Pipettes</td>
<td>Lot 1 Lot 2 Lot 1 Lot 3</td>
</tr>
<tr>
<td>Pipettor with Tips</td>
<td>Set A Set B Set A Set C</td>
</tr>
<tr>
<td>pH Meter</td>
<td>A B A B</td>
</tr>
<tr>
<td>Analytical Balance</td>
<td>1 2 2 1</td>
</tr>
<tr>
<td>Autoclave</td>
<td>1 2 3 2</td>
</tr>
<tr>
<td>Agar Tempering Water Bath</td>
<td>2 1 1 2</td>
</tr>
<tr>
<td>Incubator</td>
<td>2 3 1 5</td>
</tr>
</tbody>
</table>

Risks become ANOVA variables
# How does APLM Work?

## ANOVA TABLE

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Counts Log$_{10}$ CFU/g</th>
<th>Condition 1</th>
<th>Condition 2</th>
<th>Condition 3</th>
<th>Condition 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>Average</td>
</tr>
</tbody>
</table>

| Std. Dev. ($S_C$) | 0.1080 | 0.0841 | 0.0888 | 0.1160 |
| Variance ($S_C^2$) | 0.0117 | 0.0071 | 0.0079 | 0.0134 |
| Average (C) | 11.491 | 11.312 | 11.411 | 11.044 |

Intermediate precision = Pooled Std. Dev ($S_{IP}$)

Std. Dev. for single plate count ($S_{P_1}$) = 0.1033

SEM for average of three plate counts ($S_{P_3}$) = 0.05864

Std. Dev. for sample preparation ($S_{PREP}$) = 0.080393
### How does APLM work?

- **Is the Procedure Fit for Intended Use?**

<table>
<thead>
<tr>
<th>Performance Characteristic</th>
<th>ATP Requirement</th>
<th>Experimental Result</th>
<th>Pass (✓) / Fail (✗)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulated Specification</td>
<td>$12.962 \log_{10} \text{CFU/g}$</td>
<td>$12.962 \log_{10} \text{CFU/g}$</td>
<td>✓</td>
</tr>
<tr>
<td>Standard Uncertainty</td>
<td>$&lt; 1.215 \log_{10} \text{CFU/g}$</td>
<td>$0.827 \log_{10} \text{CFU/g}$</td>
<td>✓</td>
</tr>
<tr>
<td>Probability of Being Wrong</td>
<td>≤ 5%</td>
<td>0.85%</td>
<td>✓</td>
</tr>
</tbody>
</table>
How does APLM work?

Stage 3: Ongoing Procedure Performance Verification (OPPV)

- Ensures the analytical procedure remains in control during routine use
  - Routine monitoring
  - Analytical controls
  - Control charts
**Enumeration Sub-team’s Perspective on APLM:**

- Can be successfully applied to CFU analytical procedures for probiotics
- Addresses plate count challenges
  - Stay in control of measurement uncertainty, ensuring that the procedure is generating quality data
  - ANOVA can be used to help understand and improve uncertainty and robustness
SUMMARY

Enumeration Sub-team’s Perspective:

- The approach
  - Streamlined work while allowing flexibility
  - Drove documentation and communications surrounding the example ingredient and analytical procedure we used in our simulation
  - Maintained our knowledge base

- Information and data gathered, analyzed, and evaluated demonstrated use of sound science throughout the APLM stages and provided insights on how to make improvements

Thank You

The standard of trust
Stay Connected
Dr. Jean Schoeni | E-mail: JeanSchoeni@EurofinsUS.com

The standard of trust