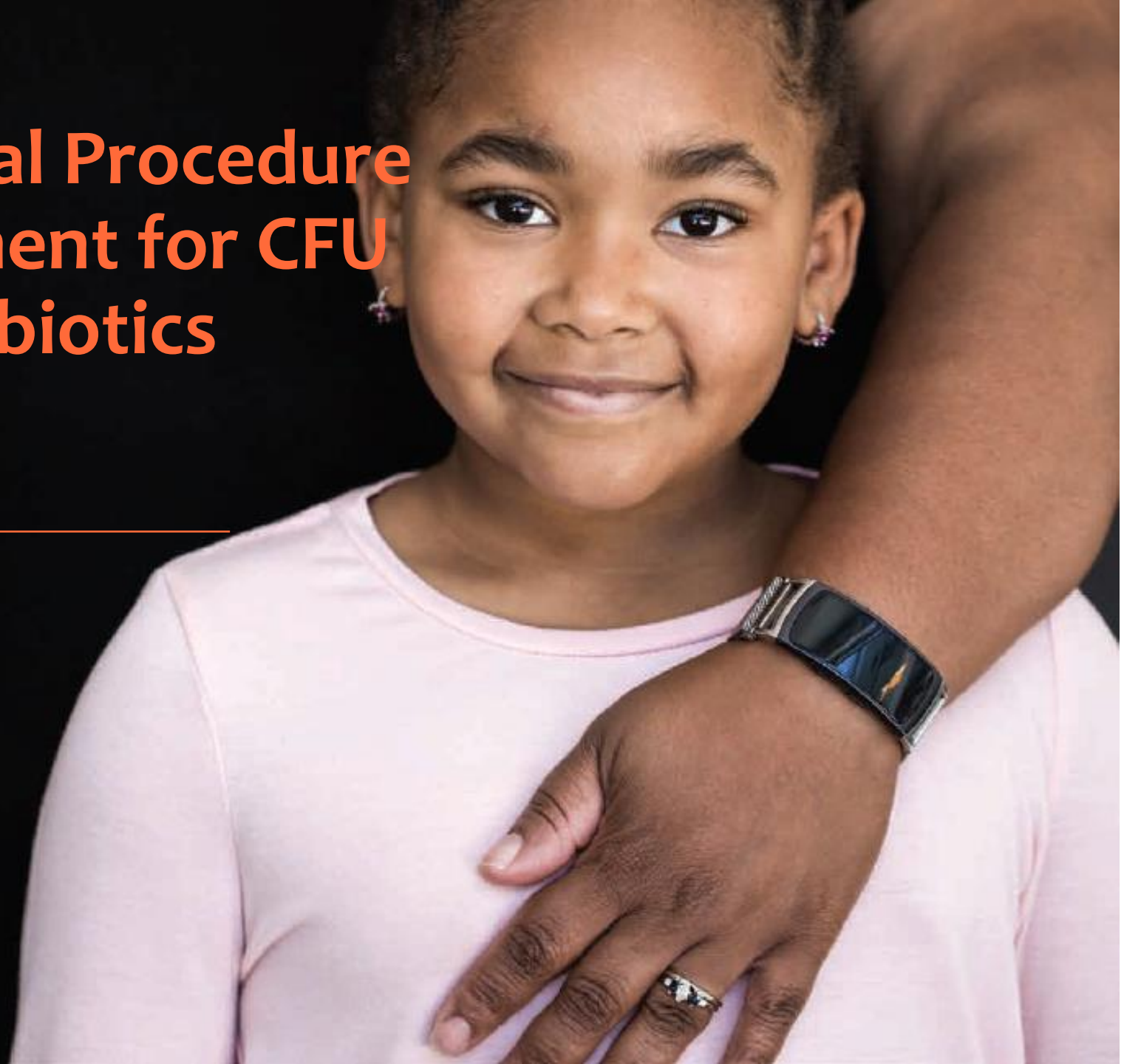
USP Open Forum

Comparing Probiotic Plate Count Methods

June 16, 2022, 10:00 am • 12:30 pm ET

Virtual Meeting

<1220> 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

200 usp The standard of trust

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

Analytical Procedure Life Cycle Management (APLM)

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

METHODS
published: 12 July 2021
doi: 10.3389/fmicb.2021.693066

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

*M. L. Jane Weitzel[1,2], Christina S. Vegge[3], Marco Pane[4], Virginia S. Goldman[5], Binu Koshy[5], Cisse Hedegaard Porsby[3], Pierre Burguière[6] and Jean L. Schoeni[7]**

Analytical Procedure Life Cycle Management (APLM)

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

Analytical Procedure Life Cycle Management (APLM)

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)**

Measurand

- 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} CFU/g, so the reportable values fall below a *TMU* = 0.305 Log_{10} CFU/g (i.e., the *TMU* associated with the reportable value is < 0.305) and the probability of being wrong is ≤ 5%. The plating range used by the laboratory will cover 9.462-13.462 Log_{10} 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?

Risk Assessment

USP GC <1220>

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

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?

Risks become ANOVA variables

UNCERTAINTY COMPONENT	CONDITIONS			
	1	2	3	4
Days	A	B	C	D
Analyst	A	B	A	C
Lot of Plating Medium	1	2	1	2
Lot of Suspension / Rehydration Medium	2	2	1	1
Lot of Dilution Buffer	1	2	3	4
Disposable Serological Pipettes	Lot 1	Lot 2	Lot 1	Lot 3
Pipettor with Tips	Set A	Set B	Set A	Set C
pH Meter	A	B	A	B
Analytical Balance	1	2	2	1
Autoclave	1	2	3	2
Agar Tempering Water Bath	2	1	1	2
Incubator	2	3	1	5

How does APLM Work?

ANOVA TABLE

Replicate	Counts Log_{10} CFU/g															
	Condition 1				Condition 2				Condition 3				Condition 4			
	1	2	3	Average	1	2	3	Average	1	2	3	Average	1	2	3	Average
1	11.336	11.478	11.424	11.416	11.236	11.443	11.311	11.330	11.335	11.575	11.234	11.381	11.162	11.326	11.211	11.233
2	11.146	11.312	11.485	11.404	11.442	11.357	11.224	11.341	11.531	11.606	11.584	11.574	10.964	10.996	10.959	10.973
3	11.506	11.688	11.583	11.592	11.466	11.274	11.348	11.356	11.418	11.373	11.386	11.392	11.169	10.946	10.945	11.020
4	11.324	11.363	11.178	11.288	11.167	11.297	11.295	11.253	11.506	11.275	11.322	11.368	10.929	11.018	11.112	11.020
5	11.397	11.519	11.358	11.425	11.424	11.267	11.416	11.369	11.351	11.315	11.282	11.316	11.206	10.986	11.093	11.095
6	11.511	11.639	11.565	11.572	11.416	11.272	11.439	11.376	11.439	11.460	11.695	11.531	10.962	10.815	10.798	10.858
7	11.436	11.510	11.503	11.483	11.511	11.338	11.446	11.432	11.446	11.546	11.441	11.478	11.154	11.290	11.071	11.172
8	11.551	11.700	11.486	11.579	11.193	11.203	11.366	11.254	11.413	11.409	11.389	11.404	11.047	11.191	11.081	11.106
9	11.429	11.607	11.521	11.519	11.283	11.276	11.265	11.275	11.334	11.563	11.018	11.305	10.870	11.005	10.819	10.898
10	11.733	11.712	11.462	11.636	11.258	10.997	11.156	11.137	11.407	11.201	11.486	11.365	10.999	11.074	11.127	11.067
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})	0.1001															
Std. Dev. for single plate count (S_{P1})	0.1033															
SEM for average of three plate counts (S_{P3})	0.05964															
Std. Dev. for sample preparation (S_{PREP})	0.080393															

How does APLM work?

- Is the Procedure Fit for Intended Use?

Performance Characteristic	ATP Requirement	Experimental Result	Pass (✓) / Fail (x)
Formulated Specification	12.962 Log_{10} CFU/g	12.962 Log_{10} CFU/g	✓
Standard Uncertainty	< 1.215 Log_{10} CFU/g	0.827 Log_{10} CFU/g	✓
Probability of Being Wrong	≤ 5%	0.85%	✓

How does APLM work?

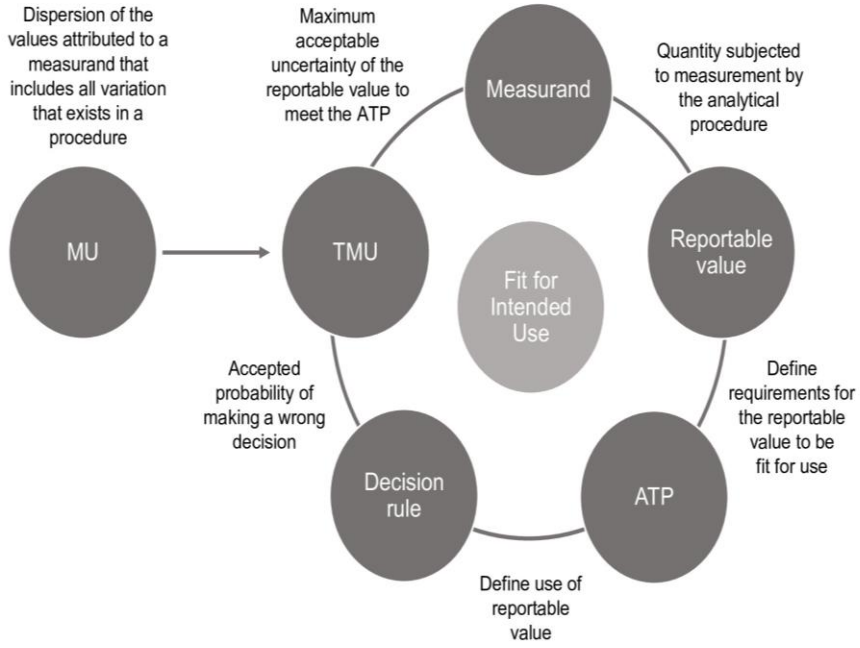
Stage 3: Ongoing Procedure Performance Verification (OPPV)

- Ensures the analytical procedure remains in control during routine use
  - Routine monitoring
  - Analytical controls
  - Control charts

Analytical Procedure Life Cycle Management (APLM)

SUMMARY

- 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

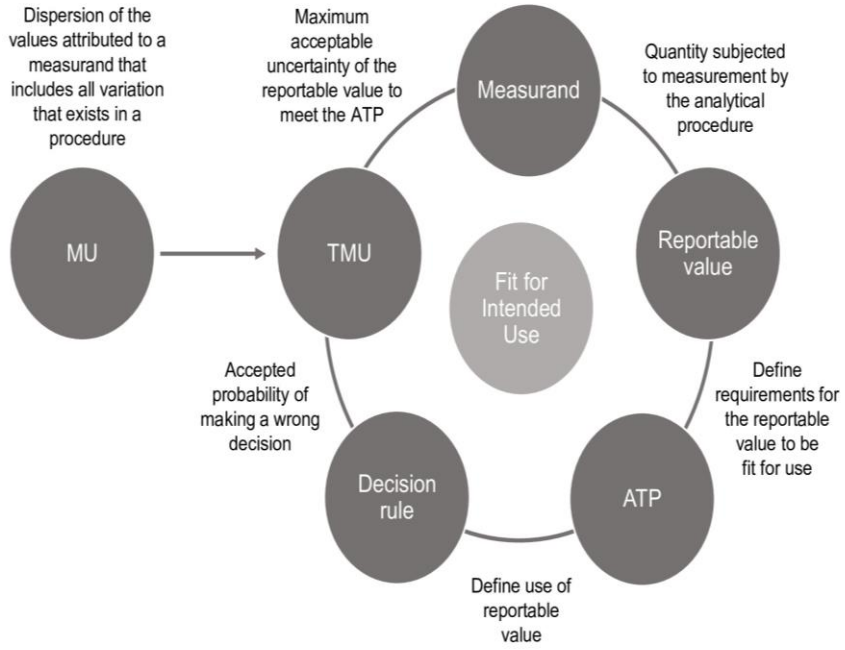


Weitzel, M.L.J., et al. 2021. doi: 10.3389fmicb.2021.693066

Analytical Procedure Lifecycle Management (APLM)

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



Weitzel, M.L.J., et al. 2021. doi: 10.3389fmicb.2021.693066

APLM and Comparisons

Thank You

The standard of trust

Stay Connected

Dr. Jean Schoeni | E-mail: JeanSchoeni@EurofinsUS.com

The standard of trust