CRN PRESENTS

LIVE Q&A: PROBIOTICS EXPERTS

WEDNESDAY, SEPTEMBER 13, 1-2 PM EASTERN

Moderator

Andrea Wong
SVP, Scientific & Regulatory Affairs | *CRN*

Dr. Wong joined CRN in 2013. She leads CRN's scientific and regulatory affairs department, responding to emerging regulatory issues, as well as advocating for science-based nutrition. Dr. Wong provides scientific expertise in evaluating research relevant to the benefits and safety of ingredients and dietary supplements, and in support of CRN's nutrition policy activities. She also leads proactive self-regulatory initiatives, including the development of best practices and guidelines for industry on product labeling and formulation. Dr. Wong has held several leadership positions, including President of the Institute of Food Technologists Washington D.C. Section. She currently serves as a member of the Institute for Organization Management Northeast Board of Regents and the U.S. Hemp Authority Board of Directors. Prior to joining CRN, she worked as a senior scientific and regulatory consultant for the firm Intertek. Dr. Wong earned her B.Sc. in Life Sciences from Queen's University and her Ph.D. in Toxicology from the University of Toronto.

Housekeeping

- This webinar is being recorded and will be available on demand on CRN's website.
- We will have Q&A after all the speakers have presented. Virtually raise your hand or put your question in the chat.
- Please stay muted unless you are asking a question.

Speakers

Jeremy Bartos, PhD
Chief Science Officer
MeriCal

Andrzej Benkowski
Technical Manager
Eurofins Center of Excellence

Shikha Snigdha, PhD
Director of Scientific Affairs
OLLY

Jeremy Bartos, PhD

Chief Science Officer | *MeriCal*

Jeremy Bartos, PhD, is the Chief Science Officer at MeriCal as well as the current Chair of CRN's Probiotic Working Group. He has over 15 years of experience working in the dietary supplements industry in a variety of R&D, product development, innovation, business development and sales roles. In his current role, he utilizes his knowledge of the scientific and clinical benefits of nutraceutical ingredients to create new finished product solutions for the dietary supplement industry, specializing in probiotic, sports nutrition, and health condition-specific innovative solutions in both dry dosage and gummy forms.

His accolades include authoring peer-reviewed publications, co-inventing numerous natural products patents and launching both branded proprietary ingredients and successful finished products tailored for various dietary supplement markets.

Dr. Bartos received his B.S. in Ecology and Evolutionary Biology from the University of Rochester and his PhD in Molecular Biology from Roswell Park Cancer Institute, a division of the State University of New York at Buffalo.

MERICAL

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The Art of Manufacturing a Dry Dosage Probiotic Supplement

Jeremy Bartos, Ph.D.
Chief Science Officer, MeriCal

WHY MANUFACTURING CONDITIONS MATTER

World Health Organization Definition of Probiotics:

- "Live microorganisms which when administered in adequate amounts confer a health benefit on the host"

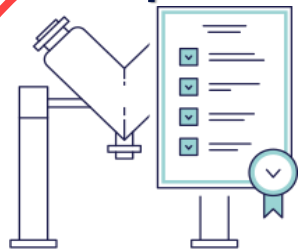
Step 1

strains delivered in refrigerated conditions

Step 2

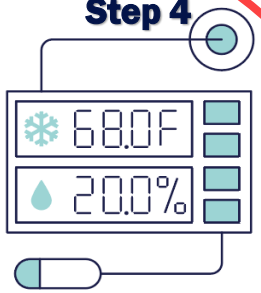
strains stored in our cold storage

Step 3



strains processed for manufacturing

Step 4



manufactured & packaged in temperature & humidity controlled conditions

Step 5

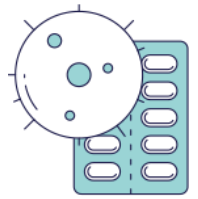
finished goods back to cold storage

Step 6

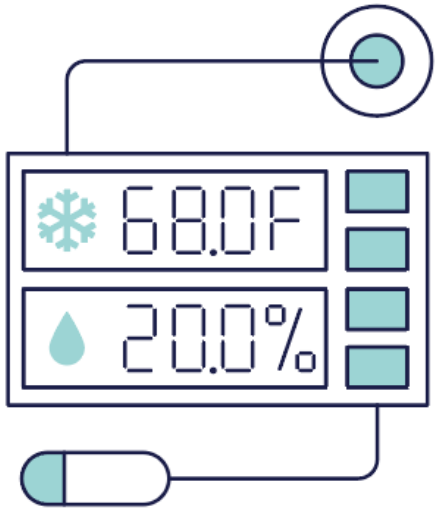
product transported to customer

KEEP THE BUGS ALIVE !!

WHY MANUFACTURING CONDITIONS MATTER

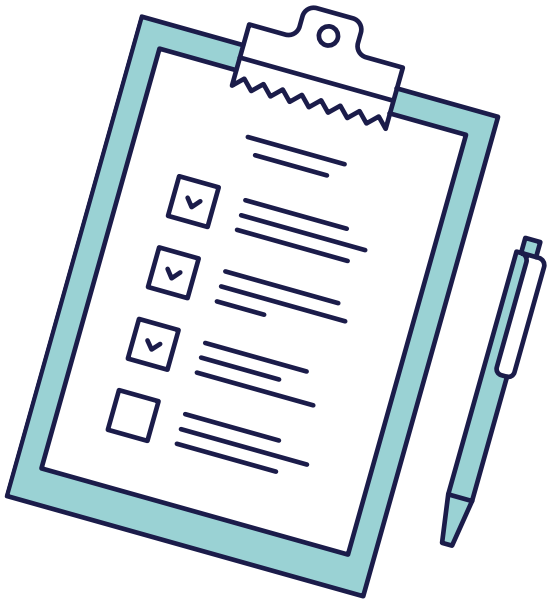


- Probiotics received “just in time” frozen and stored at 2-4°C
- Temper the probiotics at room temperature for 24 hours prior to blending
  - Minimizes risk of condensation
- Blend in a low shear mixer
  - Minimizes breakage of rod-shaped probiotics
- Desired temperature range: 15-20°C
- Desired relative humidity: as low as possible!!
  - Aim for <20% RH for all steps of the process
    - Weighing
    - Blending
    - Encapsulation/Compression
    - Packaging



CHOOSING THE RIGHT PROBIOTIC STRAINS

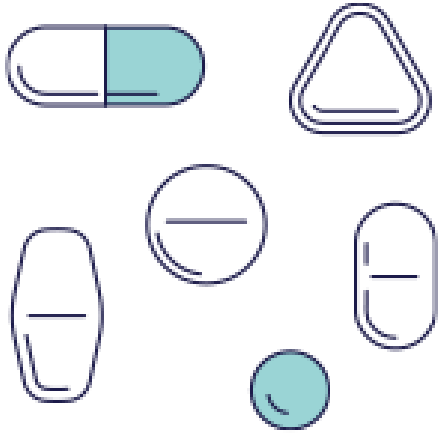
- Type of probiotic
  - “Traditional” (Lactic Acid)
  - Spore-forming
  - Yeast
- Finished product marketing claims
- Finished product CFU/AFU count
- Number of strains in the product
  - CFU count of each strain in the product
- Other ingredients in the finished product
  - Non-actives
  - Actives
    - Can affect enumeration testing
    - Can affect product stability



CHOOSING THE FINISHED PRODUCT MATRIX

- **Targeted Demographic**
  - Children: Chewables, Ready-To-Mix (RTM) powders, gummies
  - Elderly: Chewables, RTM powders, fast melt powders
  - Adult: Capsule, tablet, etc…
  - Sports Nutrition: RTM powder

- **Other ingredients combined with probiotics?**
  - Ingredient flavor profile (highly bitter/sour may be best in capsule/tablet)
  - Ingredient stability (inhibition assays)
  - Ingredient properties (hygroscopic, bulk density, etc.)



- **Formulation serving size (per piece):**
  - Capsule: <800 mg
  - Tablet: <2000 mg
  - Gummy: <400 mg
  - RTM Powder: 1 gram+

CHOOSING PACKAGING

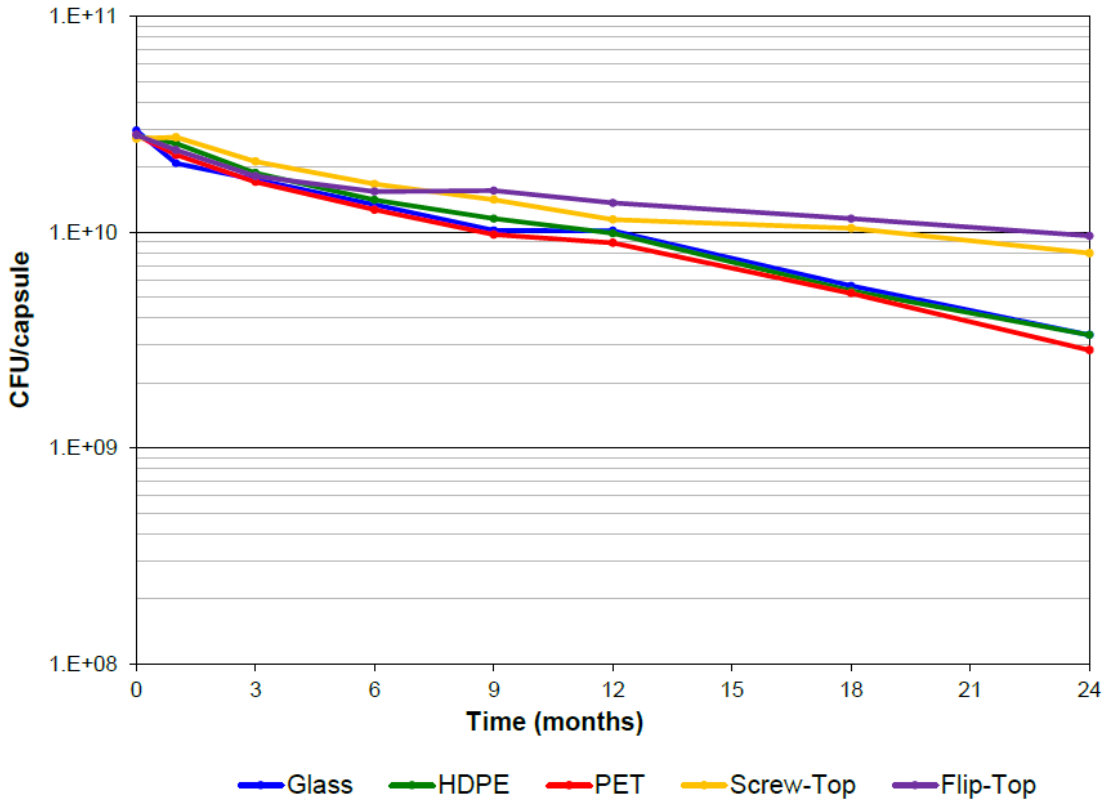
Stability is key to probiotic packaging and restricting water is key to stability!!

Stability of “Probiotic Strain A” in capsules in various bottle types:
Stored at 25C, ambient humidity

Excipient:
Avicel MCC

T zero Aw:
0.137

Format:
HPMC Capsule,
Size 0



Data provided by IFF

CHOOSING PACKAGING

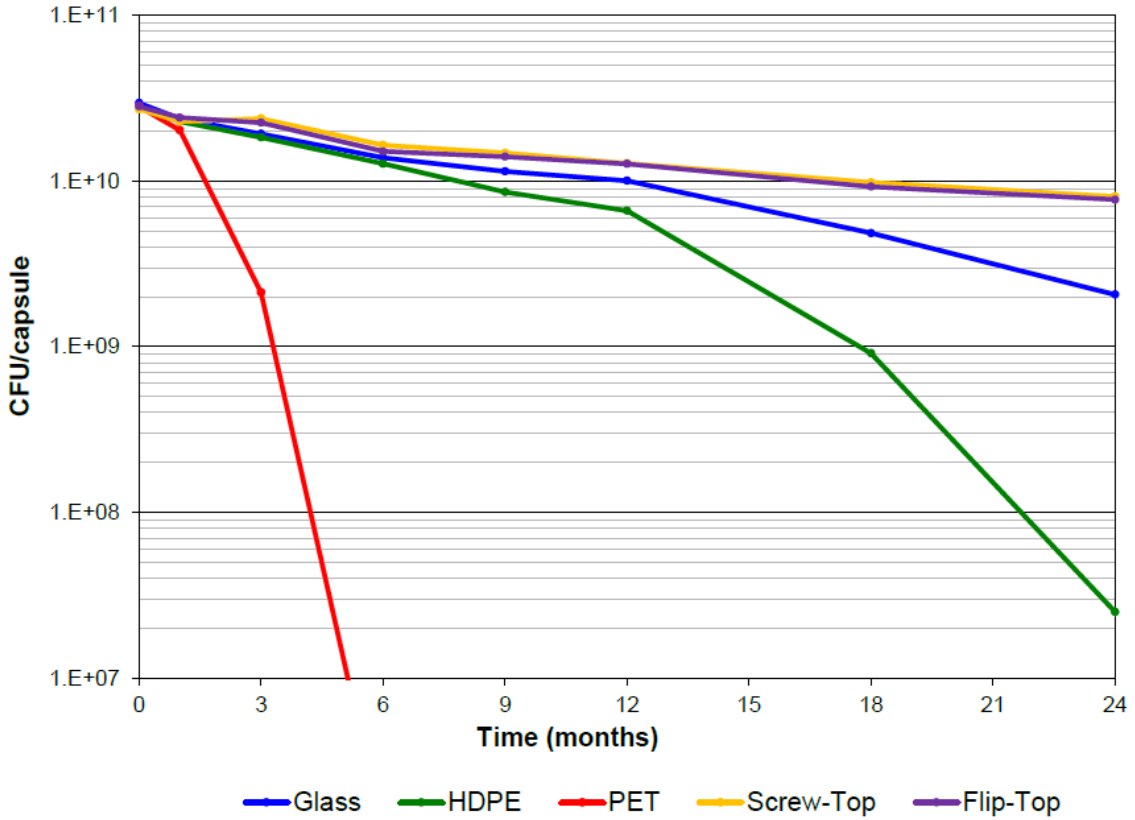
Stability is key to probiotic packaging and restricting water is key to stability!!

Stability of "Probiotic Strain A" in capsules in various bottle types:
Stored at 25C, 60% relative humidity

Excipient:
Avicel MCC

T zero Aw:
0.137

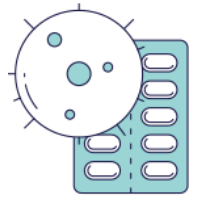
Format:
HPMC Capsule,
Size 0



- Desiccant-Lined Screw-Top/Flip-Top Vial
- Alu/Alu Blister card
- Glass
- Stick Pack (depending on foil thickness chosen)
- Alu-PVC/PVDC Blister Card
- HDPE Bottle (with desiccant)
- PET Bottle

Data provided by IFF

CHOOSING STORAGE CONDITIONS

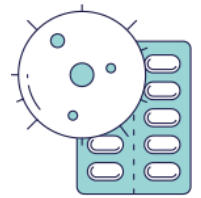


The cooler and drier the better, choose your strains wisely and choose your packaging wording wisely!

- 18-month stability study, capsules with MCC in HDPE bottle with desiccant:
  - *Lactobacillus acidophilus* strain X
    - % loss at 4C: **9%**
    - % loss at 25C: **52%**
  - *Bifidobacterium lactis* strain Y
    - % loss at 4C: **24%**
    - % loss at 25C: **74%**
  - *Lactobacillus salivarius* strain Z
    - % loss at 4C: **9%**
    - % loss at 25C: **99%**

- **"Store at 4C/refrigerated conditions":** Best stability, most difficult shipping conditions
- **"Store in cool and dry conditions":** Required to keep throughout shipping and shelf life
- **"Store at room temperature":** Easiest to ship, hardest to keep stable
- **"Store in controlled room temperature conditions":** 20-25°C (68-77°F)
- **"Store in controlled room temperature conditions. Refrigeration not necessary but may extend product shelf life":** Best of both worlds

DETERMINING REQUIRED OVERAGES

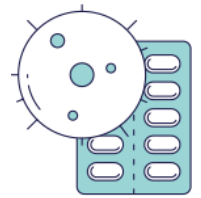


- **Manufacturing Conditions:** As cool/dry as possible
- **Probiotic species/strains:** Choose wisely, some are more stable than others
- **Non-probiotic ingredients:** Growth inhibition/interference and stability detriment
- **Finished product matrix:** Chewables/tablets will require more overages than capsules/powders due to loss during compression
- **Shelf life length/expiry:** The longer the shelf life the more overages needed
- **Storage conditions:** The warmer the storage conditions the more overages needed
- **Packaging:** Inferior packaging requires greater overages
- **Label claims:** "Through expiration" requires more overages than "At time of manufacture"

Example #1: Capsule in CSP Vial with 18-month shelf life, 3 stable strains:
150% total overages

Example #2: Chewable in HDPE bottle, 24-month shelf life, 5 stable strains, 2 non-probiotics:
450% total overages

SUMMARY



- **Manufacturing Conditions Matter:**
  - As cool/dry as possible, work with a probiotic expert when at all possible.
- **Choosing Probiotic Strains:**
  - Choose based on desired claims, stability, CFU count, and finished product matrix
- **Consider Non-Probiotic Ingredients:**
  - Be sure to determine interference before formulating
- **Choosing Finished Product Matrix:**
  - Based on probiotic strains, non-probiotic ingredients, and target demographic
- **Choose Storage Condition Wording Wisely:**
  - What you put on the package needs to be followed throughout supply chain, shipping, and shelf-life
- **Packaging Matters!:**
  - More expensive packaging options can save you on overages and stability headaches in the long run

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THANK YOU

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Andrzej Benkowski
Technical Manager | *Eurofins Center of Excellence*

Andrzej Benkowski is a Technical Manager and subject matter expert in probiotic testing based out of the Eurofins Center of Excellence for Probiotics in Madison, WI. He has over 15 years of experience in the field of microbiology specializing in contract R&D, method development, and emerging technologies for probiotic evaluation including flow cytometry and genomics platforms. Andrzej is the Chair of the International Probiotic Association Technical Committee and Co-Convenor of the ISO Working Group 11 Enumeration Subgroup.

Quality Management of Probiotics

Testing for Enumeration, Identification, & Product Safety

Andrzej Benkowski

Technical Manager

Eurofins Microbiology Laboratories - Madison, WI

Testing Probiotics – Fit for Purpose Methods

Probiotics are unique ingredients that necessitate specific approaches to testing.

Options exist for enumeration and identification.

Testing for microbiological contaminants pose challenges due to high microbial load.

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Product Potency - Enumeration

Cultural Plate Count

“Gold Standard”, Colony Forming Units (CFU)

Flow Cytometry

Cell Viability, Active Fluorescing Units (AFU)

real time PCR / digital PCR

Species/Strain specificity, Copies/µL

Plate Count Enumerations

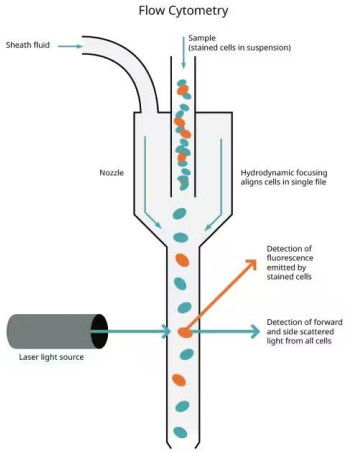
- Considerations when selecting an enumeration method.
- Proper validation/verification required.
- Common challenges include:
  - Method selection
  - Method variability
  - Challenges with recovery
  - Viability based on cultivability
  - Lack of specificity

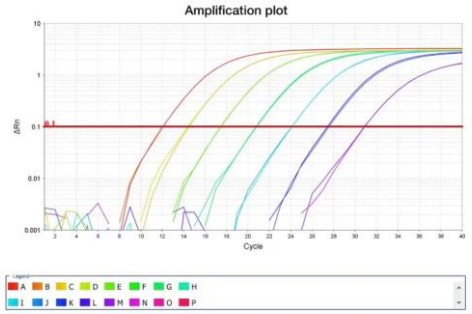
Alternatives to Plate Counts

Flow Cytometry

ISO 19344 Protocol B standard assay measures cell viability based on membrane integrity.

Live, dead and injured cell populations. Viable but not culturable (VBNC). AFU total count (AFU ≠ CFU).





qPCR/dPCR

End-point DNA-based quantification, viability with pretreatment, real-time(q) versus digital.

Power in its specificity, requires optimization and validation, tailored to analyte.

Identification of Probiotics

Strain or Species-Specific PCR

- Targeted amplification.
- Dependent on primer and hydrolysis probe design.
- Can be a challenge to distinguish different strains of the same species.

Lane	Primer Set	Strain	Product Size
M	NA	1 Kb Plus Ladder	Ladder
1	Lp-115 #1	No DNA	None
2	Lp-115 #1	No DNA	None
3	Lp-115 #1	No DNA	None
4	Lp-115 #1	No DNA	None
5	Lp-115 #1	No DNA	None
6	Lp-115 #1	No DNA	None
7	Lp-115 #1	Lp-115	165
8	Lp-115 #1	Lp-115	165
9	Lp-115 #1	Lp-115	165
10	Lp-115 #1	Lp-115	165

Metagenomic Sequencing

- Species-specific IDs in a blend.
- Next-Generation Sequencing = faster and more affordable.

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Challenges with Contaminant Testing

- High microbial load creates difficulties in finding the contaminating organism of interest.
  - Competitive inhibition
  - Needle in a haystack
- PCR-based rapid throughput methods can have limitations, but protocols exist to accommodate the unique properties of the product.
- USP Chapters <61/62> and <2021/2022> used for dietary supplement ingredients and finished products.
  - Suitability tests demonstrate method is fit for purpose.
- ISO 13559 Non-lactic contaminant method can be applied to products beyond fermented dairy with some caveats.

Key Takeaways

Probiotics are unique dietary ingredients that require special approaches to quality management when it comes to testing the materials.

Options exist for potency evaluations, each with distinct benefits and limitations. Considerations such as the matrix and organism should be taken into account when deciding how to approach quantification. The same can be said for microbiological contaminant testing.

Technologies used for the identification of probiotics species/strains continue to improve as the cost for testing decreases though challenges still exist especially when targeting strain-level ID.

Thank You!

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Shikha Snigdha, PhD
Director of Scientific Affairs | *OLLY*

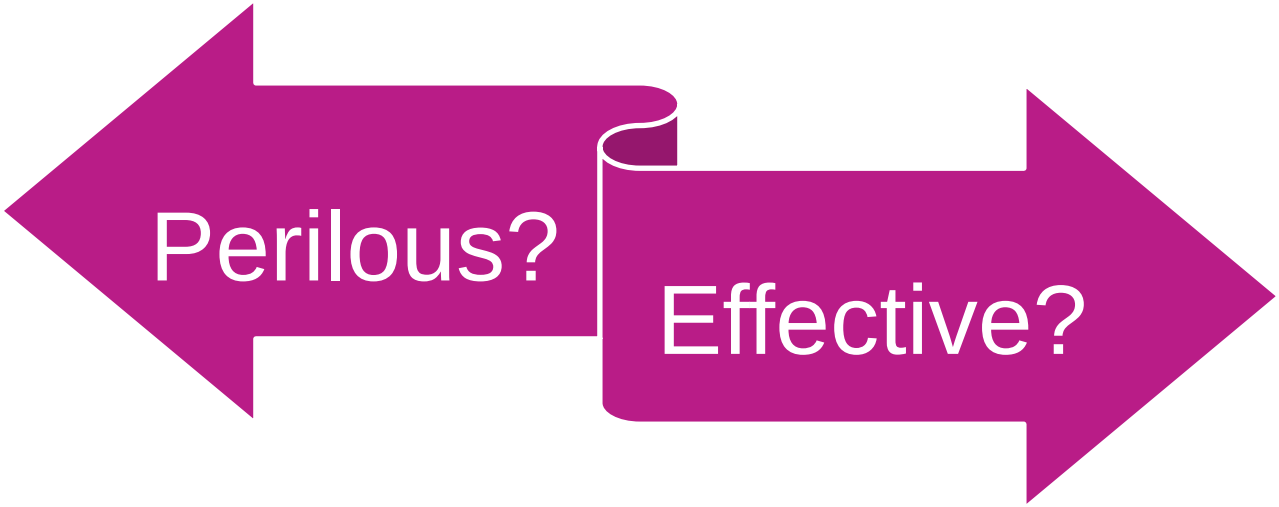
Dr. Shikha Snigdha is the Director of Scientific Affairs at OLLY, within the Unilever Health and Wellbeing Collective. She is a neuroscientist by training and a strategic leader who has been working for many years at the interface of scientific innovation and nutrition. Her work spans nutrition, dietary interventions, and the impact of gut health and microbiome on overall health and aging. She has published over 20+ manuscripts, including book chapters and holds a patent on the role of epigenetic interventions to support cognitive function and anxiety related disorders. She has a keen interest in utilizing science and innovation available to the industry to provide health equity to those who need it.

Clinical Studies in Probiotics: Separating Fact from Hype

Shikha Snigdha, Ph.d
Director Scientific Affairs

THE CASE FOR CLINICAL TRIALS

THE TRUTH ABOUT PROBIOTICS?



COMPLEXITY OF SCIENCE WITH PROBIOTICS

The Probiotic Conundrum
Regulatory Confusion, Conflicting Studies, and Safety Concerns

Stephen B. Freedman, MDCM, MSc[1,2]; David Schnadower, MD, MPH[3,4]; Phillip I. Tarr, MD[5,6]
» Author Affiliations
JAMA. 2020;323(9):823-824. doi:10.1001/jama.2019.22268

Efficient symptomatic treatment and viral load reduction for children with influenza virus infection by nasal-spraying *Bacillus* spore probiotics

Tu Thanh Tran, Thuy Thi Bich Phung ... Anh Thi Van Nguyen

Artificial-enzymes-armed *Bifidobacterium longum* probiotics for alleviating intestinal inflammation and microbiota dysbiosis

Approaches to treat inflammatory bowel disease with probiotics or artificial enzymes have advantages and limitations. Here we combine the advantages to overcome the individual limitations by modifying probiotics with artificial enzymes and demonstrate application in treating inflammatory bowel disease.

Strain-specific impacts of probiotics are a significant driver of gut microbiome development in very preterm infants

Metagenomics and metabolomics analysis of a longitudinal cohort of 123 very preterm infants reveals multiple drivers of gut microbiome development and indicates that there are strain-specific effects of probiotic products.

Perilous?

Effective?

THE TRUTH ABOUT PROBIOTICS AND SCIENCTIFIC STUDIES

NOT ALL PROBIOTICS ARE THE SAME

NOT ALL SCIENCE IS THE SAME

CRITICAL FACTORS FOR PROBIOTIC STUDIES

1. Strain Selection: Careful selection of the specific probiotic strain is crucial. Different strains can have varying effects on health.

2. Dosage: Determining the optimal dosage is essential for achieving desired health outcomes without adverse effects.

3. Duration: The study's duration should be sufficient to observe both short-term and long-term effects of probiotic consumption.

4. Control Group: Including a well-matched control group is vital to compare the outcomes with those not taking probiotics.

5. Blinding: Blinding participants and researchers reduces bias and ensures the study's integrity.

6. Outcome: The study should focus on a specific health outcome to provide meaningful results.

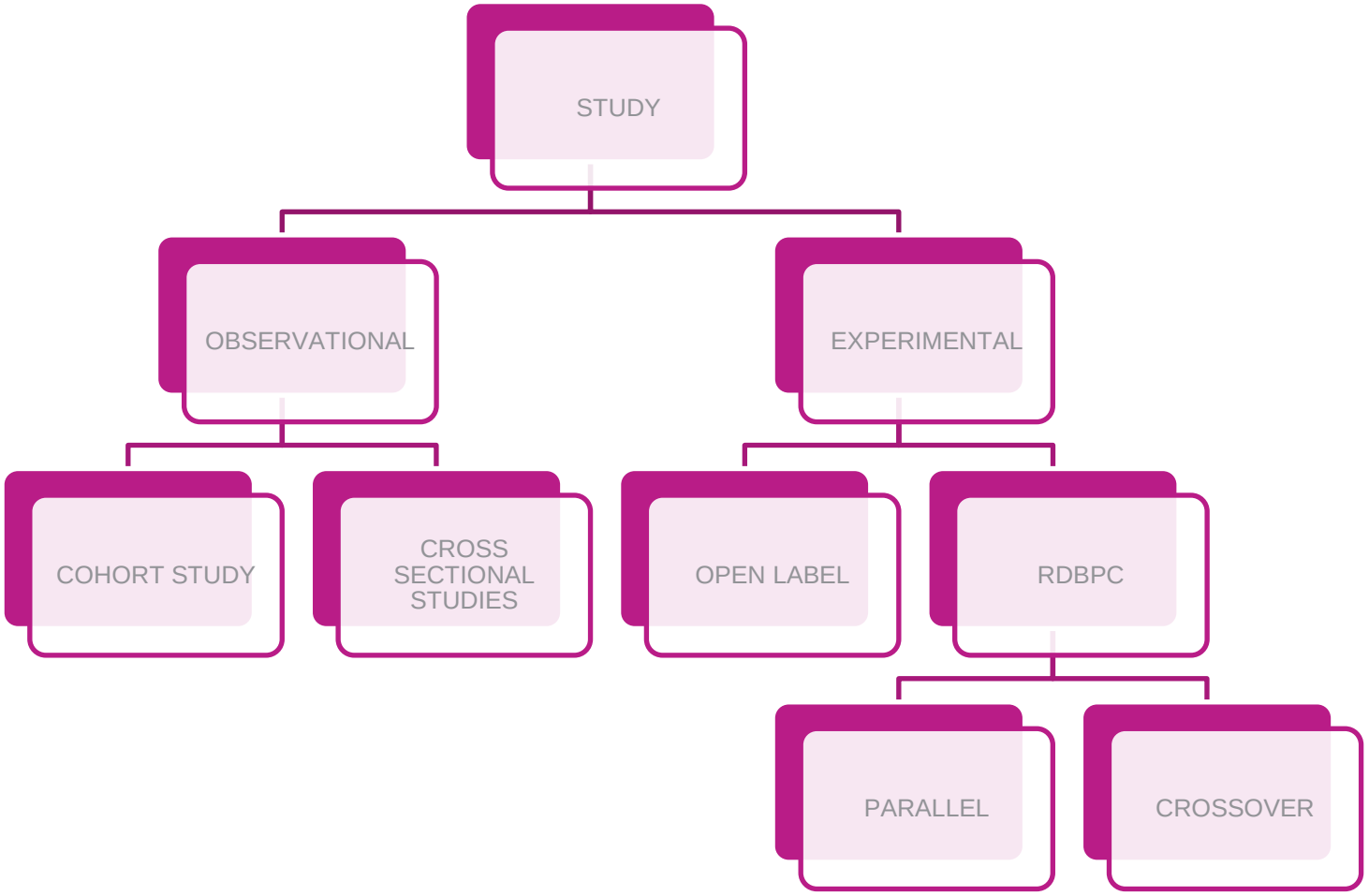
RCTs- The gold standard of clinicals

When it comes to studying the effects of substances on human health, there's a gold standard: Randomized Clinical Trials, or RCTs.

In an RCT, subjects are randomly assigned to different study groups. This randomness is key to ensuring the results are reliable and unbiased.

Blinding, where participants don't know which group they're in, adds another layer of rigor to the process.

TYPES OF CLINICAL TRIALS



WHY ARE CLINICAL TRIALS IMPORTANT

Claim and Claim Substantiation- Allows you to make valid claims on your products

Taming the Commercial Storm: Amidst flashy headlines and product assertions, clinical trials provide a grounded lens to discern truth from hype.

Informed Choices: Clinical trials empower consumers to make evidence-based decisions.

Balancing Potential with Evidence: Clinical trials act as a pivot, elevating probiotics from speculative promise to substantiated health contributions.

ADVANCES IN CLINICAL TRIALS

ARTIFICIAL INTELLIGENCE (AI) AND MACHINE LEARNING: AI IS BEING EMPLOYED TO

ANALYZE LARGE DATASETS, PREDICT SUBJECT OUTCOMES,

SIMULATE GI EFFECTS

REAL-WORLD EVIDENCE (RWE): DATA COLLECTED FROM REAL-WORLD SETTINGS, SUCH AS ELECTRONIC HEALTH RECORDS

VIRTUAL CLINICAL TRIALS AND WEARABLES

CENTERS OF RESEARCH FOR PROBIOTICS

1. **University of California, Davis - Foods for Health Institute:** Advancing knowledge at the intersection of food, nutrition, and health with collaboration opportunities for new ingredients, products and services.

1. **Harvard Medical School – Microbiome in Public Health Center:** Research using cohort samples/platform for discovery, validation, and translation of novel therapeutics derived from the microbiome.

2. **University of Wisconsin-Madison - Food Research Institute:** Exploring the potential benefits of probiotics and their role in food safety and quality.

3. **Massachusetts Institute of Technology (MIT) - Center for Microbiome Informatics and Therapeutics:** Innovating in microbiome research with 'partner with us' initiatives.

4. **University of California, San Diego - Center for Microbiome Innovation:** Advancing microbiome technology and research, including probiotics with lab to market initiatives.

CONCLUSION

- Clinical trials allow for evidence-based decisions for product design
- They are essential for the development and regulation of dietary supplements.
- They provide valuable information on the safety and effectiveness of these products
- Clinical trials are evolving- we should leverage these changes

LLY | THANK YOU!

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WEDNESDAY, SEPTEMBER 13, 1-2 PM EASTERN