USP Open Forum

Comparing Probiotic Plate Count Methods

June 16, 2022, 10:00 am - 12:30 pm ET
Virtual Meeting
USP Approach to Probiotic Enumeration

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Zooming in on Probiotics

Product

- Blend of lyophilized probiotic bacteria and excipients

Content

- Viable lyophilized bacterial cells

Dietary ingredient

Photo from ISAPP
https://isappscience.org/tag/freeze-drying/
Probiotic potency

Potency by CFUs

Colony Forming Units (CFU) on agar plate

Enumeration of viable cells

Supplement Facts

<table>
<thead>
<tr>
<th>Amount Per Serving</th>
<th>% Daily Value</th>
<th>% Daily Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>1 g</td>
<td>&lt;1%**</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>0 g</td>
<td>**</td>
</tr>
<tr>
<td>Ind. &amp; Added Sugars</td>
<td>0 g</td>
<td>0%</td>
</tr>
<tr>
<td>Vitamin D (as cholecalciferol)</td>
<td>10 mcg</td>
<td>8%</td>
</tr>
</tbody>
</table>

Proprietary Blend: Lactobacillus rhamnosus GG (LGG®), Bifidobacterium animalis subsp. lactis (BB-12®), 2’-Fucosyllactose

18 mg

Total Cultures: (3.5 billion CFUs)

Other Ingredients: Maltodextrin

May contain traces of milk (lactose).
Can all bacterial cells form a CFU?

Viable cells able to form a CFU

Membrane intact cells measured by flow cytometry

Correlation to CFU dosage used in clinical trials
CFU: Golden standard for probiotic potency
## Plate count methods in USP

<table>
<thead>
<tr>
<th>Chapter/Monograph</th>
<th>Plate count method</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic Tests &lt;64&gt;</td>
<td>Enumeration assay for non-spore-forming bacteria strains</td>
<td>Official</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em></td>
<td>&lt;64&gt;</td>
<td>Official</td>
</tr>
<tr>
<td><em>Lactobacillus reuteri</em></td>
<td>&lt;64&gt;</td>
<td>Official</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>&lt;64&gt;</td>
<td>01Aug2022</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em></td>
<td>&lt;64&gt;</td>
<td>01Aug2022</td>
</tr>
<tr>
<td><em>Lacticaseibacillus casei</em></td>
<td>&lt;64&gt;</td>
<td>01Aug2022</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em></td>
<td>&lt;64&gt; modified (agar)</td>
<td>Official</td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em> subsp. lactis</td>
<td>&lt;64&gt; modified (agar, incubation)</td>
<td>Official</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em> subsp. longum</td>
<td>&lt;64&gt; modified (agar)</td>
<td>Official</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em> subsp. infantis</td>
<td>&lt;64&gt; modified (agar, sample preparation)</td>
<td>01Aug2022</td>
</tr>
<tr>
<td><em>Bacillus coagulans</em></td>
<td>Specific method for <em>Bacillus coagulans</em></td>
<td>Official</td>
</tr>
<tr>
<td><em>Bacillus clausii</em></td>
<td>Specific method for <em>Bacillus clausii</em></td>
<td>Official</td>
</tr>
<tr>
<td>Additional monographs under way</td>
<td>Unknown</td>
<td>Pending</td>
</tr>
</tbody>
</table>
General challenges of plate count methods

Ideal sample preparation

Type and shelf life of agar

Enumeration of multiple strains with different growth requirements

Time consuming - up to 6 days

Robustness of method

No reference standards

Uncertainty of method

Comparison of methods
Impact of method uncertainty

USP Acceptance criteria:
≥ 100% of the labeled viable cell count (cfu/g)
How to manage CFU methods

How to evaluate and control the method uncertainty?

How to compare the performance of two different methods?

How to evaluate the risk of a false OOS?

How to evaluate the impact on changing to a new supplier of agar?

How many replicates are needed?
Thank You

The standard of trust
Stay Connected
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