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Joint Committee on Dietary Supplements

April 10, 2024

Proposed revision to NSF/ANSI 173 – Dietary Supplements (173i110r2)

Revision 2 of NSF/ANSI 173, issue 110 is being forwarded to the Joint Committee for consideration. Please review the proposal and **submit your ballot by May 1, 2024** via the NSF Online Workspace.

Please review all ballot materials. When adding comments, please include the section number applicable to your comment and add all comments under one comment number whenever possible. If you need additional space, please use the attached blank comment template in the reference documents and upload online via the browse function.

Please note that your last recorded vote from any previous ballot draft revision(s) will not be carried forward. Please respond affirmative, negative, or abstain to the content of this revision. Comments on any prior revision(s) will not be carried forward.

Purpose

The proposed is to amend NSF/ANSI Standard 173, Section 4.2 (Probiotics) to permit the use of well-validated alternatives to colony forming units (CFU) in reporting the number of viable bacteria in a probiotic ingredient or product. This change would recognize the utility of relatively new, direct methods of enumeration, such as those employing fluorescence-based flow cytometry, as opposed to the traditional plate count method.

Background

The r1 of this issue was balloted at the end of 2023. It received 3 negative votes. After that ballot those members who voted negative as long with some observers who are very knowledgeable in this field met several times to workshop a new version. This ballot is the result of those meetings.

For over 100 years, plate count enumeration has been the standard method for quantifying viable bacterial cells. Plate counting is straightforward: serial dilutions of cells are prepared, spotted onto an appropriate medium, and incubated under appropriate conditions for growth, until the resulting colonies can be seen and counted. The accompanying unit of measure, the CFU, is a useful indicator of the biological potency of a sample or product. However, recent technological advances have enabled the development of new and more direct methods for quantification of live bacterial cells,

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which may offer substantial advantages over traditional plate count-based methods.

Chief among these methods is flow cytometric enumeration, which generally relies on fluorescent stains selective for live versus dead bacterial cells and provides readouts in terms of active fluorescent units (AFU) rather than CFU. Flow cytometric methods, such as those described in ISO 19344 IDF 232, can offer greater precision than plate count enumeration, which has a relative standard deviation of 10-15% and is susceptible to factors such as cellular aggregation: when several bacterial cells are clumped together (as occurs naturally for non-motile organisms reproducing by binary fission), they are counted as a single CFU in plate count enumeration unless they are separated by fluid shear in the process of sample preparation. Whether or not this separation occurs may depend heavily on the species and strain of bacterium in question, the media and other growth conditions, and even the amount of force with which a technician pipettes and mixes the sample during preparation.

In principle, plate count enumeration and CFU readings are also susceptible to antagonistic interactions among strains: many microbes produce soluble bacteriocins, small molecules, and other factors which may inhibit the growth of nearby colonies on a plate, in a way that does not necessarily reflect their activity under biologically relevant conditions. Furthermore, recent evidence suggests that as bacterial cells age in stasis, they may enter a “viable but nonculturable” state, which exhibits metabolic activity but does not necessarily reproduce to form colonies.

While some issues with plate-count enumeration can theoretically be solved by adaptations to traditional methods, others may be insurmountable without the adoption of new technology. For instance: most mammals, including many humans, possess gut bacteria which convert cholesterol into the insoluble compound coprostanol, allowing it to be excreted in stool rather than absorbed in the small intestine. This has the net effect of reducing the host’s blood cholesterol levels, and the rate of coprostanol production by a person’s gut microbiome is inversely associated with their risk of both heart disease and colorectal cancer. Naturally, this is an appealing target for development of a novel probiotic—however, most coprostanol-producing strains appear to be obligately planktonic, i.e. they can only reproduce in liquid culture, and cannot be grown as colonies on solid media. If such a strain is one day developed as a probiotic, products containing it will be ineligible for NSF certification given the current wording of NSF/ANSI Standard 173.

By directly examining and enumerating cells as they exist in the product as consumed, flow cytometric methods can circumvent these irregularities and inconsistencies and provide consumers with a fuller and more accurate picture of a product’s biological activity—in addition to a more precise one. For manufacturers and regulators, novel enumeration methods can provide advantages in terms of reproducibility and throughput, especially in the case of probiotics containing multiple organisms that have different fastidious growth requirements. Lastly, the adoption of AFU as a measure of viability offers a new vista in the assessment and validation of shelf life potency through expiry.

To date, CFU has been adequate as a tool for standardizing probiotics—but it is

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important to consider that, to date, nearly all probiotic strains have come from a relatively narrow range of taxa. We have only just begun to tap the potential of the human microbiome, and the probiotics of the future will likely contain organisms from all across the kingdom Bacteria. This is a taxonomic group as broad and diverse as Eukarya, which encompasses everything from the ant to the quaking aspen—so it should come as no surprise that the diversity of form and function these microbes embody will require us to adapt the way we think and communicate about them. It will require the invention and adoption of new techniques, and new terms to better reflect our growing knowledge.
If you have any questions about the technical content of the ballot, you may contact me in care of:

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[Note – the recommended changes to the standard which include the current text of the relevant section(s) indicate deletions by use of ~~strikeout~~ and additions by grey highlighting. Rationale Statements are in *italics* and only used to add clarity; these statements will NOT be in the finished publication.]

NSF/ANSI Standard
for Health Sciences –

Dietary Supplements

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4 Labeling and Literature Requirements

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4.2 Probiotics

For products and ingredients containing probiotics, the following information ~~must~~ shall be present on the label:

— minimum CFU count, viable cell count (expressed according to the method used), or a combination of both, of each strain of live microorganism at the time of the product or ingredient's expiration, or at time of production if no expiration date is applied; or

— minimum total CFU count, viable cell count (expressed according to the method used), or a combination of both, for a blend of live microorganisms at the time of the product or ingredient's expiration, or at time of production if no expiration date is applied; and

— potency values depicted on a probiotic-containing ingredient or product are consistent with the test(s) used by the company in establishing their specifications; and

— storage directions that guarantee the minimum CFU count(s), viable cell count(s), or a combination of both, at the time of expiration, or at time of production if no expiration date is applied; and

— identification of the probiotic including genus, species, and strain based on widely accepted nomenclature. If a trademarked name is used to identify the bacteria, the genus, species, and strain should also be included on the label; and

— finished products offered for sale in the USA must first list the quantity of a strain or blend of strains in terms of weight (e.g. milligrams); the labeling regulations of other jurisdictions outside of the USA shall take precedence over those offered herein.

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