

Appendix to the 2017 Request for Pre-Proposals for Research on Sampling & Sample Preparation for Microbiological Food Testing

Drawing an accurate conclusion about whether a product (e.g. ingredient or finished) is safe based on the result of a test is important to the evaluation and management of food safety risk. Test results that reflect the real level of risk are predicated on a number of governing factors that provide legitimacy to the data. It is these principal factors that provide the basis for meaningful test results. These factors include: sample collection procedures sample frequency and number, sample size, sample preparation and test method used.

Maximizing the probability of finding a target hazard in an ingredient, processing environment, or a finished product with heterogeneous and low level contamination is essential to the credibility of the data upon which food safety decisions are made. Two critical areas that enhance probability are the efficient and statistically representative collection of samples from bulk product and sample preparation procedures that optimize the detection of pathogens in a variety of food matrices.

When quantitative indicator organism assays are performed to assess the level of process control and hygiene in a manufacturing facility, the same need for representative sampling exists.

While specific deliverables are detailed within the scope of the Requests for Pre-proposal, additional considerations important in the production of food are outlined below:

Sample Considerations:

- I. The sample must be representative of the product in question
- II. Sample Size
- III. Pooling/Compositing
 - a. [Guidance Document for Lotting and Sampling of Beef Products for Pathogen Analysis](#)
 - b. International Commission on Microbiological Specifications for Foods (ICMSF) recommendations and guidelines
- IV. Types of Media
- V. Enrichment Techniques
- VI. Positive Control(s) for the Sample
 - a. Nonpathogenic, if possible
 - b. Easy to use
 - c. Low count for qualitative methods
- VII. Sample Type Characteristics
 - a. Background Flora – What level is this at? Will the background flora interfere or inhibit the method? For example: chicken coop floor swabs, raw meat, fermented foods (cheese, yogurt, etc.), probiotic culture containing foods, fecal samples, raw milk, etc.



- b. % Fat – Can the fat be homogenized for a representative sample? How will the fat be kept in suspension/homogenized? For example: ground beef, vegetable oils, spreads, etc.
 - c. Moisture or Water Activity – Will high salt or high sugar content interfere with the method? For example: salted meats, flour, peanut butter, chocolate, dried fruit, sweeteners, etc.
 - d. Solubility or Viscosity – If the sample does not dissolve in or mix with water, can something be added to help and not interfere with the method? Will it last for the entire time needed? For example: polystyrenes, sterols, gums, starches, compressed pellets of feed, etc.
 - e. Color – Will the color, especially dark colors interfere with the method? For example: sauces, dressings, cocoa, chocolate, fruit concentrations, colorants, spices, etc.
 - f. pH – Will a highly acidic sample or highly basic sample need to be adjusted and if so will that change the sample's behavior in the method? For example: concentrated citrus juice, vinegar, etc.
 - g. Gas – Will the gas interfere with the method and will it change over time giving what effect on the method? For example: carbonated beverages, leavening agents, etc.
 - h. Spice content – Will the level and type of spice(s) affect the method? For example: spice mixes, marinated meats, sauces, etc.
 - i. Sample make up such as polyphenol in red fruits and wines, enzymes in red offal, raw mollusks, and nuts, and molecular inhibitors found in cocoa, coffee, egg yolk, preservatives, etc.
- VIII. Recovery of Target Organism in the Matrix - Will the sample interfere with recovery of the target organism(s)? Are the organisms injured? For example: the sample is clumpy, the sample contains chunks, the sample contains non-soluble material, frozen, refrigerated, high acid, processed, raw, etc.
- IX. Inhibition of the Matrix to the Technology
- X. Limits that restrict the use of this organism in the factory lab
- XI. Non-homogeneity of matrix and target organism. for example: shells
- XII. Chemical carryover – Does the sample contain a chemical that will interfere with the method? For example: environmental swabs/sponges after sanitizing, in process samples with processing aides such as hydrogen peroxide or hexane, etc.
- XIII. Freeze-dried foods
- XIV. Dealing with samples that are likely to have a lot of dead cells in them
- XV. Environmental sampling- using appropriate collection device to the sample size, correct transport medium, what is on the sample that could interfere with the method, will the method be able to retrieve/pull out the cells from the sampling device? etc.
- XVI. Sample prep can affect confirmation – Will confirmation be able to be completed as designed?



Reading List:

Below is a list of references for further education on what affects sample preparations, what work has previously been done on sample prep, and what has been identified as issues with samples and sampling prep, etc.

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USDA, Federal Grain Inspection Service. Grain Inspection Handbook Book 1 – Sampling. July 2013. https://www.gipsa.usda.gov/fgis/handbook/BK1/BookI_2015-09-18.pdf