An Interlaboratory Study to Evaluate the Equivalence of Milk Protein Allergen Measurement

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Abstract:

- An interlaboratory study is planned by NIST to evaluate the equivalence of milk protein allergen measurements.
- The study will include participation from the manufacturers of milk protein ELISA kits, routine testing labs that utilize ELISAs, and users of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for milk protein measurement.
- The study will utilize simple samples prepared from commercial non-fat dry milk (NFDM), chosen to minimize the influence of sample extraction and sample matrix on between-method measurement equivalence.
- Planned samples include solutions of NFDM, heat-treated NFDM, irradiated NFDM, and NFDM spiked with both casein and whey proteins.
- These samples have been prepared at NIST and are being evaluated by NIST for fitness for the study.

Table 1. Candidate interlaboratory study sample

<table>
<thead>
<tr>
<th>Candidate Study Sample</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>NFDM</td>
<td>500 ppm NFDM (in PBS, pH 8.2)</td>
</tr>
<tr>
<td>Irradiated NFDM</td>
<td>500 ppm irradiated NFDM (in PBS)</td>
</tr>
<tr>
<td>100 °C heat-treated NFDM</td>
<td>500 ppm 100 °C heat-treated NFDM (in PBS)</td>
</tr>
<tr>
<td>175 °C heat-treated NFDM</td>
<td>500 ppm 175 °C heat-treated NFDM (in PBS)</td>
</tr>
<tr>
<td>NFDM + Casein</td>
<td>500 ppm NFDM + 100 ppm casein (in PBS)</td>
</tr>
<tr>
<td>NFDM + α-lactalbumin</td>
<td>500 ppm NFDM + 100 ppm α-LA (in PBS)</td>
</tr>
<tr>
<td>NFDM + β-lactoglobulin</td>
<td>500 ppm NFDM + 100 ppm β-LG (in PBS)</td>
</tr>
</tbody>
</table>

Materials and Methods:

Materials. Commercial non-fat dry milk (NFDM) was obtained from a local grocery store. Casein (technical grade, product # N7078-50G) was obtained from Sigma Life Science. Certified reference materials of bovine α-lactalbumin (product # L-045-100MG) and β-lactoglobulin (product # L-046-100MG) were obtained from Cerilliant. Candidate study materials were prepared in an aqueous phosphate-buffered saline (PBS), pH 8.2. All candidate study samples were prepared gravimetrically. Treatment of the NFDM powder was done at 100 °C for 1 h and 175 °C for 30 min using a laboratory oven. An aliquot of NFDM powder was irradiated at NIST (15 kGy of combined β- and γ-radiation from 137Cs). Table 1 lists the candidate study samples and their descriptions.

Methods. Characterization of the candidate study samples was performed using SDS gel electrophoresis (Figure 1), LC-MS/MS analysis after trypsin digestion (Figure 2), and measurement using a commercial total milk ELISA (Figure 1).

Study Design:

- Multiple aliquots of each study sample will be shipped to study participants on cold packs for preliminary testing and final study measurement.
- Study participants can use any platform (ELISA or LC-MS/MS) available to them; ELISAs for specific milk proteins or protein classes can also be used.
- Participants will use their own extraction method (if necessary) and diluents; extra sample aliquots will be provided to optimize dilution for each measurement platform.
- Study participants will report measurement results in the reporting units of their assay; additional protein-specific quantitative information can also be reported.
- Study results will be uploaded through a reporting website; NIST will perform statistical analysis on the study data and will produce a study report for publication.
- If requested, participating laboratories and assay platform identification will be blinded in the study report.
- Future studies, using more complex food-matrix samples, are also planned.

Study Timeline:

- Participant sign-up deadline = November 22, 2019
- Sample delivery deadline = December 2019
- Sample testing deadline = March 16, 2020
- Preliminary study results reporting = September 2020 (9th AOAC Annual Meeting, Orlando, FL)
- Study report publication = December 2020

For more information or to participate in the Milk Interlaboratory Study, please email: nistfoodallergens@nist.gov

Please provide:

- Names of platforms you intend to use
- Contact information
- Shipping information


Figure 1. (top) silver-stained SDS gel electrophoresis image from the analysis of candidate study samples; (bottom) normalized total milk concentrations of candidate study samples measured using a commercial total milk ELISA.

Figure 2. Comparison of the targeted LC-MS/MS (QToF) extracted ion chromatograms for 11 tryptic peptides from bovine milk proteins for the candidate study samples.