

# 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.

## Materials and Methods:

**Materials.** Commercial non-fat dry milk (NFDM) was obtained from a local grocery store. Casein (technical grade, product # C7078-500G) was obtained from Sigma Life Science. Certified reference materials of bovine alpha-lactalbumin (product # L-045-100MG) and beta-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  $\beta$ - and  $\gamma$ -radiation from <sup>60</sup>Co). Table 1 lists the candidate study samples and their descriptions.

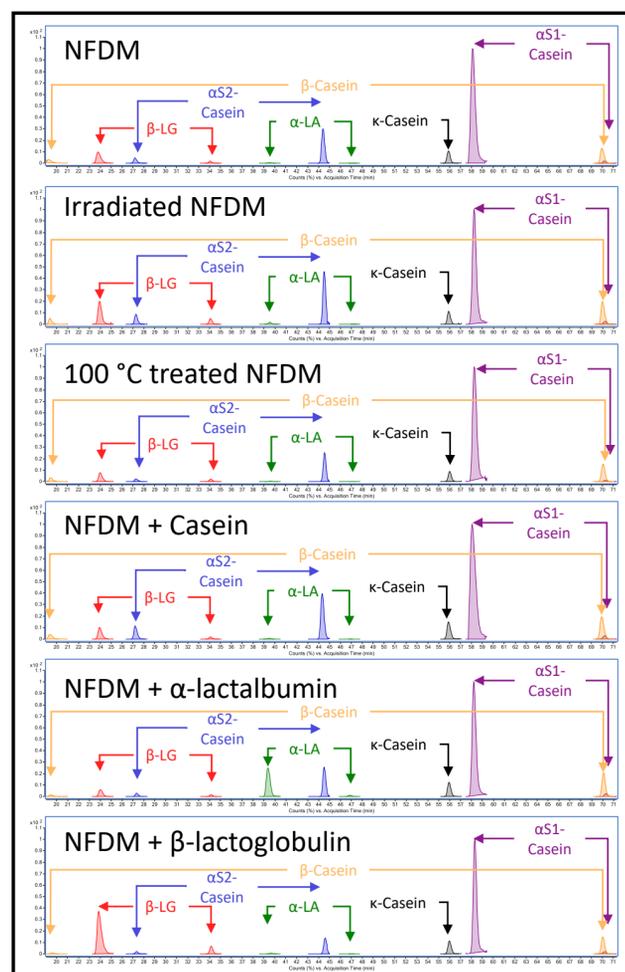
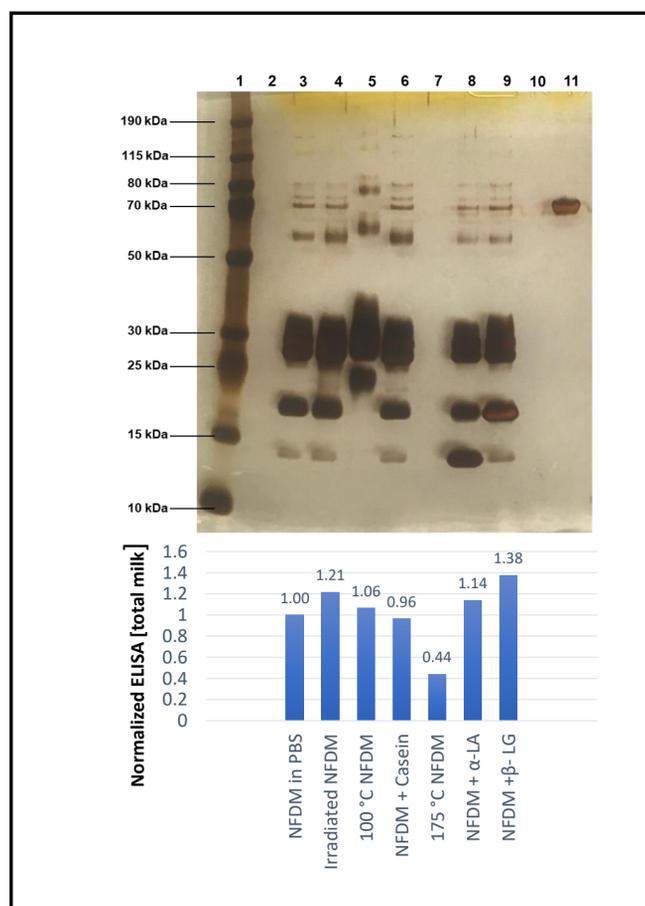
**Methods.** Characterization of the candidate study samples was performed using SDS gel electrophoresis (Figure 1), LC-MS/MS analysis after trypsin digestion (Figure 2), and measurement using a commercial total milk ELISA (Figure 1).

**Table 1.** Candidate interlaboratory study sample

Candidate Study Sample	Description
NFDM	≈ 500 ppm NFDM (in PBS, pH 8.2)
Irradiated NFDM	≈ 500 ppm irradiated NFDM (in PBS)
100 °C heat-treated NFDM	≈ 500 ppm 100 °C heat-treated NFDM (in PBS)
175 °C heat-treated NFDM	≈ 500 ppm 175 °C heat-treated NFDM (in PBS)
NFDM + Casein	≈ 500 ppm NFDM + ≈ 100 ppm casein (in PBS)
NFDM + $\alpha$ -lactalbumin	≈ 500 ppm NFDM + ≈ 100 ppm $\alpha$ -LA (in PBS)
NFDM + $\beta$ -lactoglobulin	≈ 500 ppm NFDM + ≈ 100 ppm $\beta$ -LG (in PBS)

## 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 assays; 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



**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.

## Study Timeline:

- Participant sign-up deadline = November 22, 2019
- Study sample shipment = mid-December 2019
- Result reporting deadline = March 16, 2020
- Preliminary study results reporting = September 2020 (@ 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](mailto:nistfoodallergens@nist.gov)

### Please provide:

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

<https://www.nist.gov/programs-projects/nist-food-protein-allergen-program>

