National Institute of Standards & Technology

Report of Investigation

Reference Material 8445

Spray-Dried Whole Egg for Allergen Detection

This Reference Material (RM) is intended primarily for use in evaluating test kits for determination of the presence of allergenic egg proteins. This material provides a common matrix to the allergen research community, who may wish to conduct studies using a single broadly available material. RM 8445 was prepared by representatives from the Food Allergy Research and Resource Program (FARRP), Food Products Association (FPA), Health Canada, Institute for Reference Materials and Measurements (IRMM), and U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN). This group is the sole authority for all of the information provided in this report, including the reference value and other technical information. A unit of RM 8445 consists of a single bottle containing 5 g of material.

Reference Mass Fraction Value: A reference mass fraction value for protein is provided in Table 1. A reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with an associated uncertainty that may not include all sources of uncertainty. The reference value in this material is the mean of three measurements; the associated uncertainty is the expanded uncertainty at the 95 % level of confidence [2,3]. Values are reported on an "as-received" basis in mass fraction units [4].

Expiration of Value Assignment: RM 8445 is valid, within the measurement uncertainty specified, until **15 June 2024**, provided the RM is handled and stored in accordance with the instructions given in this report (see "Instructions for Storage and Use"). The report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM Value Assignment: The characterizing agencies will monitor representative samples of this RM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

The protein in RM 8445 was determined by the Department of Food Science and Technology, University of Nebraska (Lincoln, NE).

Technical and support aspects involved in the preparation and issuance of this RM were coordinated by K.E. Sharpless, currently in the NIST Special Programs Office.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this RM were coordinated through the NIST Office of Reference Materials.

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NOTICE AND WARNING TO USERS

Warning: For laboratory use only. Not for human or animal consumption. This material is meant to serve as a reference material for comparing analytical methods with the following understanding: (1) that the use of eggs from different sources may have different compositions, and (2) if the material is subjected to various forms of preparation/processing, (i.e., baking, boiling, frying) the composition and properties of the material may change.

INSTRUCTIONS FOR STORAGE AND USE[1]

Storage: This RM, in an unopened container, should be stored at −20 °C in a desiccator or sealed plastic container that contains desiccant.

The unopened bottle should be allowed to warm to room temperature (23 °C ± 2 °C) immediately prior to use. The egg powder should not be irradiated or treated in any way that might alter the proteins present. A homogeneous stock solution containing an egg powder concentration of 100 mg/mL in phosphate-buffered saline (PBS) should be prepared using a Potter-Elvehjem tissue grinder. This stock solution should be further diluted in PBS for use in evaluation of test kits and treated as a sample according to recommended extraction procedures. Samples containing <1 μg/mL egg powder not subjected to extraction may require the addition of an additive such as 0.1 % Tween–20 or bovine serum albumin.

PREPARATION AND ANALYSIS

Source and Preparation of Material: Spray-dried whole egg powder (Henningsen Foods, Omaha, NE) without additives or stabilizers was packaged by FDA CFSAN in plastic, screw-cap bottles, each containing approximately 5 g of material.

Analysis: Nitrogen was measured using a Leco protein analyzer (Dumas) on two sets of samples, with analysis of sets separated by several months. Results were converted to protein using a conversion factor of 6.25.

Table 1. Reference Mass Fraction Value for Protein in RM 8445[a]

	Mass Fraction (%)
Total Protein	48 ± 1

[a] The reference concentration value, expressed as a mass fraction on an as-received basis, is the mean of two sets of analyses for nitrogen by the University of Nebraska's Department of Food Science and Technology. The uncertainty in the reference value, calculated according to the method described in the ISO/JCGM Guides [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the within- and between-set component of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence. The measurand is the total mass fraction of total protein as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass fraction in percent on an as-received basis.

[1] Certain commercial equipment, instruments, or materials are identified in this report in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/srm/upload/SP260-136.PDF (accessed Mar 2019).

[2] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Mar 2019); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Mar 2019).

[3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.-K.; Vangel, M.G.; Yen;, J.H.; Zhang, N.F.; *An Approach to Combining Results From Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105; p. 571 (2000).

[4] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/pubs/sp811/index.cfm (accessed Mar 2019).

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Users of this RM should ensure that the Report of Investigation in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.