Contributions of the NIST Cryogenic Reference Material Production Facility

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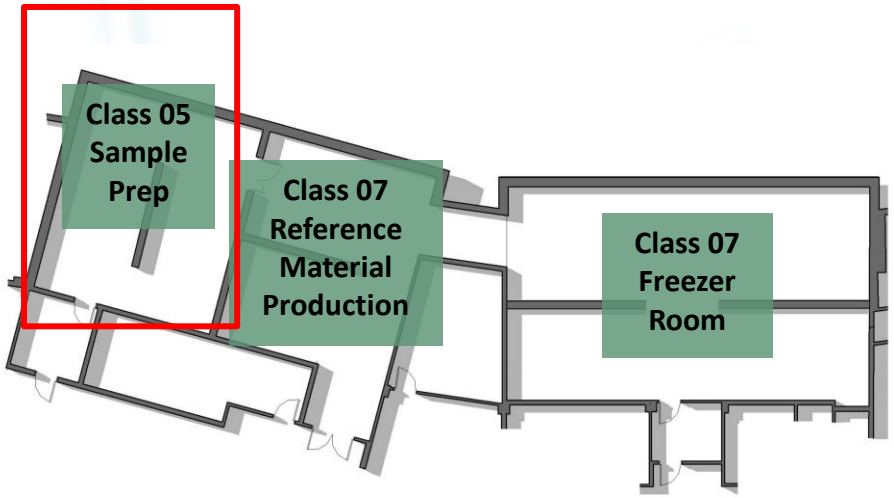
646.06 Biospecimen Science Group
Isotope Metrology Working Group Seminar
20 June 2023

NIST CHARLESTON

- HML opened in 2002
- Joint facility with 5 partner institutions
- ~ 20 NIST researchers across 4 groups
  - Inorganic and organic metrology
  - Proteomics and metabolomics
  - Data sciences
  - Biospecimen sciences & RM production

- ISO Class 5 cleanroom
  - Limit contamination of materials
- Chemical fume hoods
  - Limit exposure to users
- Fluid Dispensing
- Small-batch cryogenic homogenization equipment
- Custom amendment of cryogenic materials

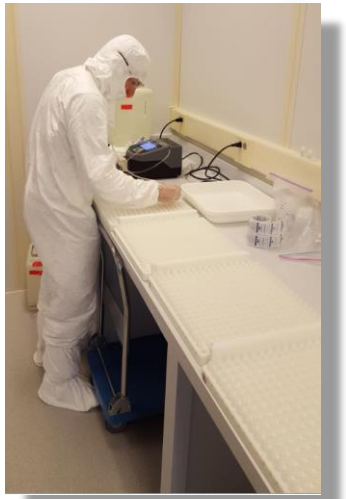


ISO 14644-1 Cleanroom Standards

Class	maximum particles/m^3						FED STD 209E equivalent
	>=0.1 µm	>=0.2 µm	>=0.3 µm	>=0.5 µm	>=1 µm	>=5 µm	
ISO 1	10	2					
ISO 2	100	24	10	4			
ISO 3	1,000	237	102	35	8		Class 1
ISO 4	10,000	2,370	1,020	352	83		Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1,000
ISO 7				352,000	83,200	2,930	Class 10,000
ISO 8				3,520,000	832,000	29,300	Class 100,000
ISO 9				35,200,000	8,320,000	293,000	Room Air

RM Production Projects

- SRM 2783a Air Particulate on Filter Media*
  - Common and toxic elements in air
- RGM 10166 Produced Water Material*
  - Hydraulic fracturing water
- RM 8301 Boron Isotopes in Marine Carbonate
  - Simulated coral and foraminifera solutions
- RGM 10122 Metabolomics System Suitability Sample (Tissue Extract)*



* in production

RM Production Projects

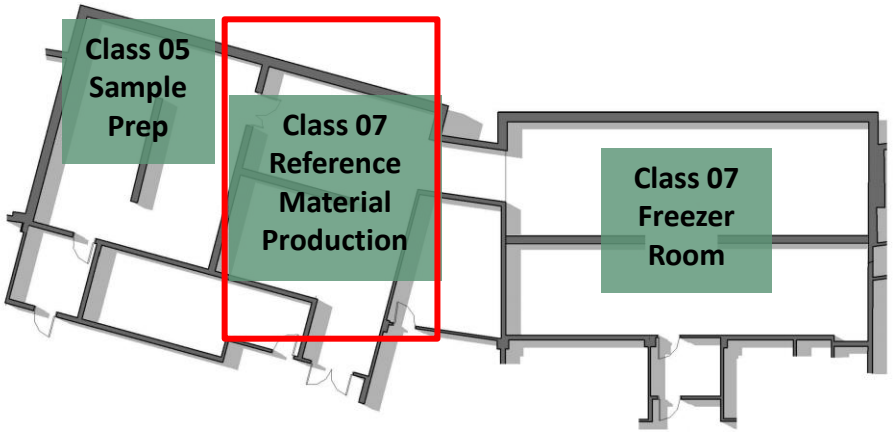
- Small batch Materials
  - RM 8048 Human Fecal Material*
    - Gut microbiome
  - RM 8462 Liver Suite (Omics Measurements Suitability)*
    - Differential metabolite and protein analysis

- Custom Amendment
  - RGTM 10190 Pesticide Residues in Frozen Spinach Leaves

* in production

FACILITIES

- ISO Class 7 Cleanroom
  - Cryogenic Reference Material Production Facility (CRMPF)
- Large batch material processing
  - Homogenization
  - Blending
  - Sieving
- Particle Size Determination



LARGE BATCH PROCESSING

RM 8695: Per- and Polyfluoroalkyl Substances in Bovine Tissue (Beef Bull)*

SRM 1974c: Organics in Mussel Tissue

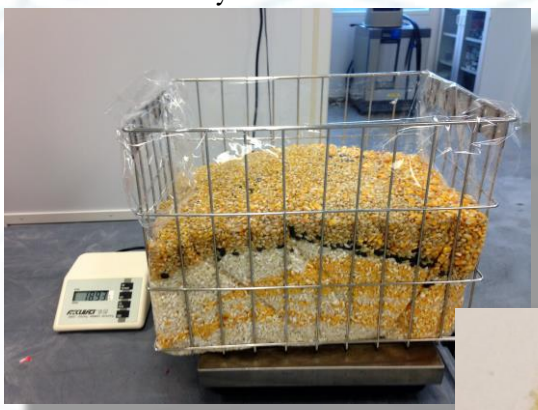
SRM 3233: Fortified Breakfast Cereal

RM 8667: Ashwagandha Root Powder

* in production

PARTICLE SIZE ANALYSIS

RM 1565: Mycotoxins in Corn



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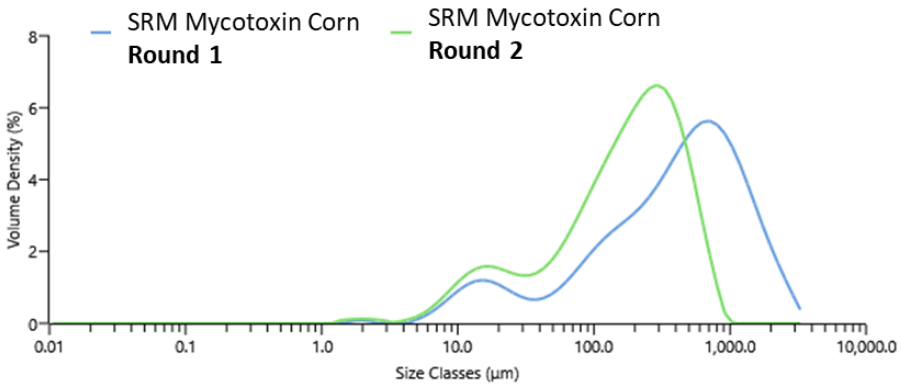


RM Production Projects

- RM 1565 Mycotoxins in Corn

- SRM 1947a Great Lakes Fish Tissue*
  - Environmental contaminants, PFAS

- SRM 3223 Inorganic Constituents in Cigarette Tobacco Filler*

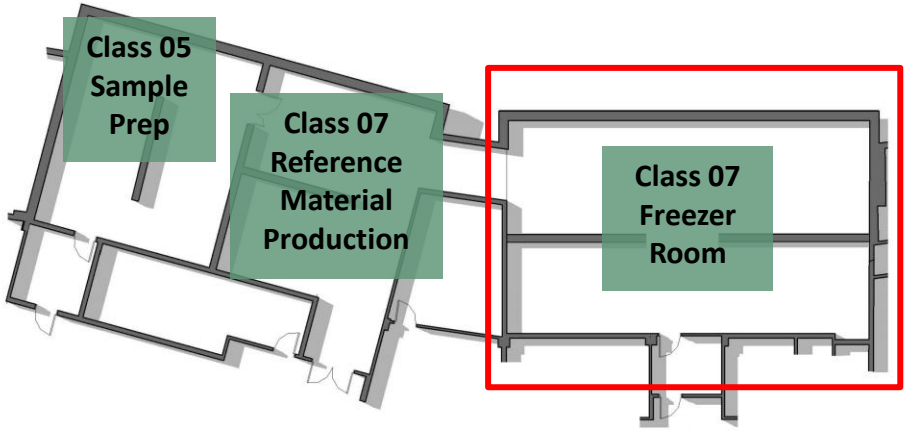
- PSA Work
  - SRM 1632e Trace Elements in Coal (Bituminous)
  - SRM 2706 New Jersey Soil, Organics and Trace Elements

* in production

FACILITIES

- ISO Class 7 Cleanroom
  - NIST Biorepository
- 25 LN_2 vapor phase freezers
- 12 −80 °C freezers

Alex Holt



RATIONALE

Problem:

- Seafood is one of the most highly-traded international commodities and is priced at import based on weight, species and provenance
- Falsification can have negative economic and human health impacts
- The majority of seafood consumed in the US is imported

Regulations:

- Food Safety Modernization Act
- Seafood Import Monitoring Program

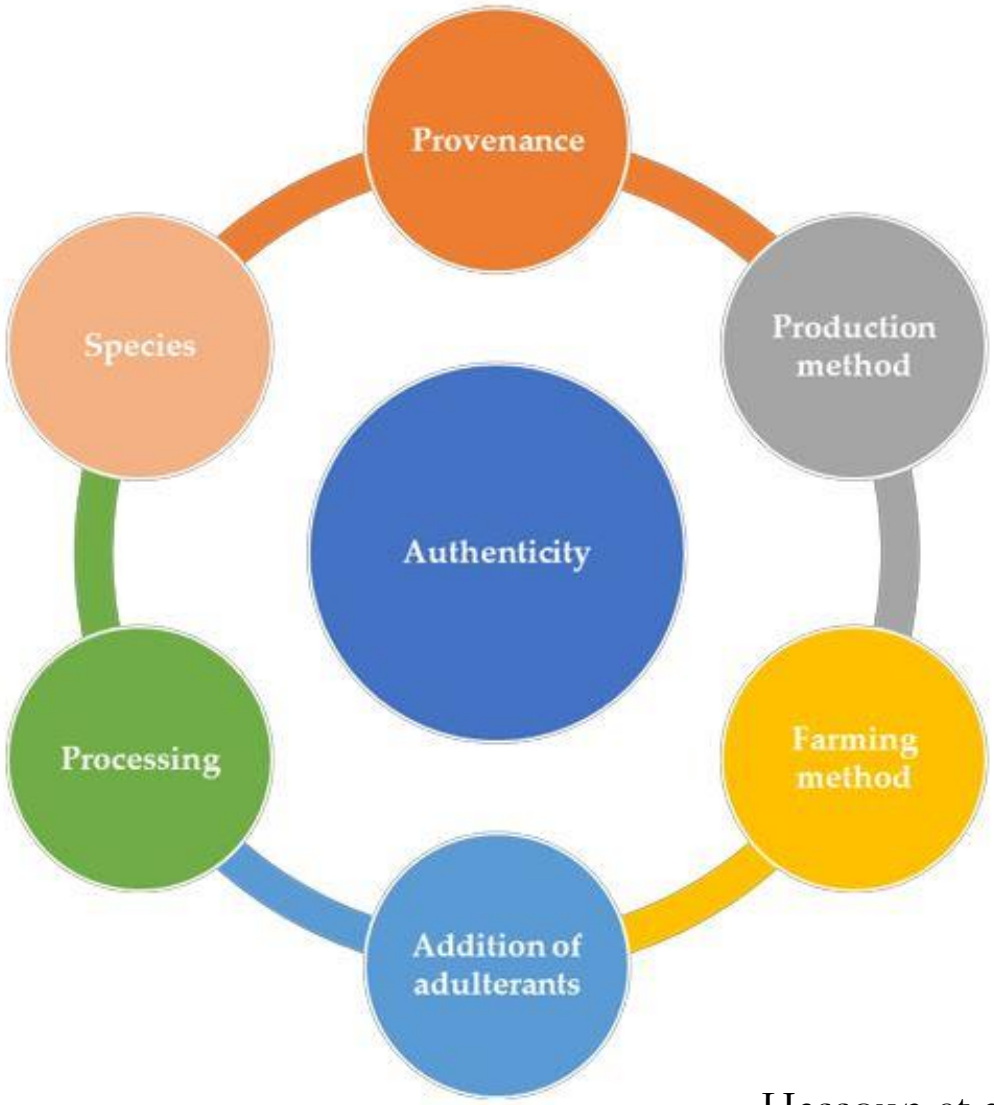
NIST's involvement:

- Provide matrix-based RMs to aid the food industry and testing laboratories in food nutrient and contaminant determinations to help ensure food safety

U.S. Customs and Border Protection

FDA U.S. FOOD & DRUG ADMINISTRATION

AUTHENTICITY



Hassoun et al, 2020

Salmon Materials

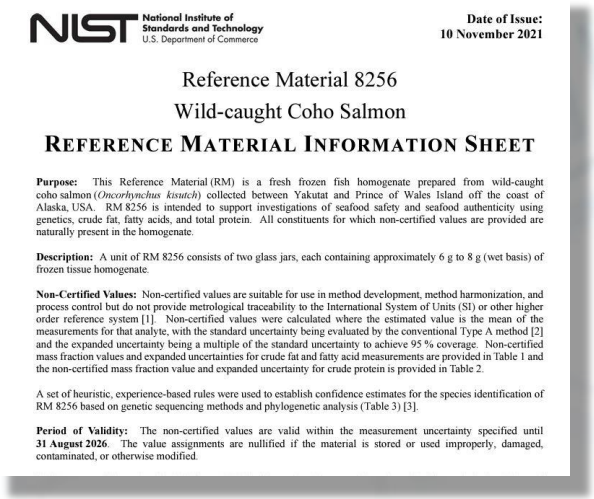
- **NIST RM 8256**: Wild caught *O. kisutch* caught ~15 mi off the coast of AK
- **NIST RM 8257**: Aquacultured *O. kisutch* sourced from river-based facility in WA

Shrimp Materials

- **NIST RM 8258**: Wild caught *F. aztecus* caught off the coast of SC
- **NIST RM 8259**: Aquacultured *L. vannamei* from land-based facility in AL

ANALYSIS

- Genetic determination (Sanger sequencing and NGS)
- Crude fat and fatty acids
- Total protein



NIST National Institute of Standards and Technology U.S. Department of Commerce

Date of Issue:
10 November 2021

Reference Material 8256
Wild-caught Coho Salmon

REFERENCE MATERIAL INFORMATION SHEET

Purpose: This Reference Material (RM) is a fresh frozen fish homogenate prepared from wild-caught coho salmon (*Oncorhynchus kisutch*) collected between Yakutat and Prince of Wales Island off the coast of Alaska, USA. RM 8256 is intended to support investigations of seafood safety and seafood authenticity using genetics, crude fat, fatty acids, and total protein. All constituents for which non-certified values are provided are naturally present in the homogenate.

Description: A unit of RM 8256 consists of two glass jars, each containing approximately 6 g to 8 g (wet basis) of frozen tissue homogenate.

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher order reference system [1]. Non-certified values were calculated where the estimated value is the mean of the measurements for that analyte, with the standard uncertainty being evaluated by the conventional Type A method [2] and the expanded uncertainty being a multiple of the standard uncertainty to achieve 95 % coverage. Non-certified mass fraction values and expanded uncertainties for crude fat and fatty acid measurements are provided in Table 1 and the non-certified mass fraction value and expanded uncertainty for crude protein is provided in Table 2.

A set of heuristic, experience-based rules were used to establish confidence estimates for the species identification of RM 8256 based on genetic sequencing methods and phylogenetic analysis (Table 3) [3].

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **31 August 2026**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

- Omics for differential analysis
  - NMR and LC-HRMS/MS
- Stable isotope analysis*

* in production

THANKS

Alex Holt