Date of Issue:
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Standard Reference Material® 1565
Mycotoxins in Corn
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended for validation of methods for determining mycotoxins in corn and similar materials.

Description: A unit of SRM 1565 consists of one bottle each of two materials: low level or blank corn and incurred corn with native levels of mycotoxins. Each bottle contains approximately 60 g of ground corn.

Certified Value: A certified value is provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST.

A certified mass fraction value for ochratoxin A in SRM 1565, reported on an as-received basis, is provided in Table 1. This value was assigned based upon measurements made at NIST and the United States Food and Drug Administration (FDA) using established methods [1–3] and includes data from collaborating laboratories participating in a NIST-conducted interlaboratory study [4]. This value is traceable to the SI measurement unit for chemical mass fraction, expressed as nanograms per gram.

Table 1. Certified Mass Fraction Value for Ochratoxin A in SRM 1565 Mycotoxins in Corn (Incurred)[(a)]

Measurand	Mass Fraction (ng/g)
Ochratoxin A	9.4 ± 1.2

[(a)] Certified values are expressed as $x \pm U_{95}(x)$, where x is the value and $U_{95}(x)$ is the expanded uncertainty of the value. The true value of the measurand lies within the interval $x \pm U_{95}(x)$ with 95 % confidence. A certified value can be regarded as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [5].

Expiration of Certification: The certification of **SRM 1565** is valid, within the measurement uncertainty specified, until **01 August 2024**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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Steven J. Choquette, Director
Office of Reference Materials

Non-Certified Values: Non-certified values are listed in Tables 2 and 3 for additional mycotoxins in SRM 1565. These non-certified values are the best current estimates based on available data but they do not meet the NIST criteria for certification. The associated uncertainties may not include all sources of bias and variability. These values were derived from results reported by NIST and/or FDA using established methods [1–3] and include data from collaborating laboratories participating in a NIST-conducted interlaboratory study [4]. These values are traceable to the measurement processes and standards used by NIST, FDA, and the study participants.

Table 2. Non-Certified Mass Fraction Values for Mycotoxins in SRM 1565 (Blank)[a]

Measurand	Mass Fraction (ng/g)
Deoxynivalenol[b,c,d]	142 ± 36
Fumonisin B_1[c,d]	10.4 ± 3.9

[a] Assigned values are expressed as $x \pm U_{95}(x)$, where x is the value and $U_{95}(x)$ is the expanded uncertainty of the value. The best estimated value of the measurand lies within the interval $x \pm U_{95}(x)$ with 95 % confidence. The assigned value can be regarded as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [5].
[b] NIST ID-LC-MS/MS
[c] FDA ID-LC-MS/MS
[d] Collaborating laboratories reporting methods as described in Appendix B.

Table 3. Non-Certified Mass Fraction Values for Mycotoxins in SRM 1565 (Incurred)[a]

Measurand	Mass Fraction (ng/g)
Aflatoxin B_1[b,c,d]	7.5 ± 1.7
Aflatoxin B_2[b,c,d]	1.43 ± 0.34
Aflatoxin G_1[b,c,d]	0.98 ± 0.19
Aflatoxin G_2[b,d]	0.87 ± 0.24
Total Aflatoxins[b,d]	10.2 ± 2.9
Deoxynivalenol[b,c,d]	467 ± 67
Fumonisin B_1[c,d]	805 ± 190
Fumonisin B_2[c,d]	217 ± 30
Fumonisin B_3[c,d]	99.3 ± 8.4
Total Fumonisins[c,d]	1150 ± 169
HT-2 Toxin[c,d]	38.2 ± 6.0
T-2 Toxin[c,d]	18.4 ± 4.2
Zearalenone[b,c,d]	61 ± 36

[a] Assigned values are expressed as $x \pm U_{95}(x)$, where x is the value and $U_{95}(x)$ is the expanded uncertainty of the value. The best estimated value of the measurand lies within the interval $x \pm U_{95}(x)$ with 95 % confidence. The assigned value can be regarded as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [5].
[b] NIST ID-LC-MS/MS
[c] FDA ID-LC-MS/MS
[d] Collaborating laboratories reporting methods as described in Appendix B.

SUPPLEMENTAL INFORMATION

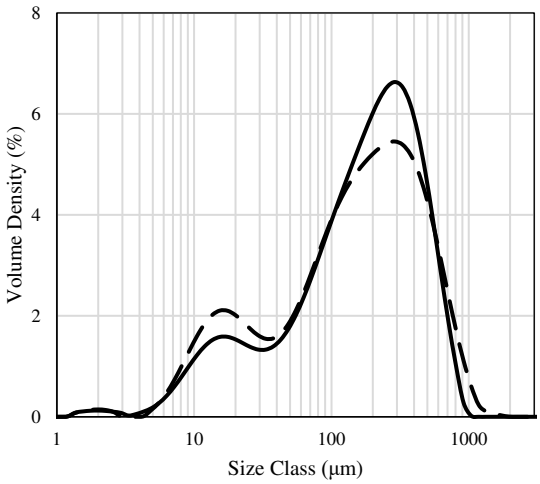

Figure 1. The mean particle sizes of the blank and incurred corns were evaluated using a Malvern Mastersizer 3000 (Westborough, MA). Corn samples (approximately 0.5 g) were dispersed in ethanol and all measurements were background corrected. The average particle size for SRM 1565 (Blank) (solid line) was determined to be 588 µm with a standard deviation of 17 µm (n = 10). For SRM 1565 (Incurred) (dashed line), the average particle size was determined to be 676 µm with a standard deviation of 92 µm (n = 10).

Storage and Handling: The original unopened bottles of SRM 1565 should be stored at room temperature (20 °C ± 5 °C). An open bottle can be reused until the material reaches its expiration date, provided that the open bottle is resealed and stored at room temperature (20 °C ± 5 °C).

Use: Before use, the contents of the unopened bottle should be mixed thoroughly by inverting and/or rolling. Homogeneity of the material has not been evaluated for sample sizes smaller than those used by NIST or FDA methods described below. Therefore, the certified and non-certified values may not be valid for test portions smaller than those described in the subsequent sections: 1 g for determination of fumonisin B_1, fumonisin B_2, fumonisin B_3, deoxynivalenol, zearalenone, T-2 toxin, and HT-2 toxin, and 5 g for determination of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, total aflatoxins, and ochratoxin A. Results obtained should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 6.

Safety: SRM 1565 is intended for research use; not for human consumption.

Source and Preparation: The SRM is a blend of ground corns. Approximately 70 kg of blank whole kernels of corn were collected from various markets in Maryland. Approximately 14 kg of homogenized ground contaminated corn were collected from FDA Office of Regulatory Science, Center for Food Science and Nutrition (CFSAN), US Department of Agriculture Federal Grain Inspection Service (USDA FGIS, Kansas City, MO), and from Trilogy Labs (Washington, MO). All materials were transferred directly from the FDA/CFSAN (College Park, MD) to the NIST Cryogenic Reference Material Production Facility (CRMPF, Charleston, SC). Whole kernels were cryohomogenized twice in a Palla STC Vibrating Cryomill (KHD Humboldt Wedag, Cologne, Germany) maintained at –180 °C for the duration of the process [7]. The contaminated corn (14 kg) was mixed with 28 kg of homogenized blank corn using a rotating blender then packaged in 60 g aliquots into amber bottles. The particle size distributions of the final packaged units of SRM 1565 were evaluated and are described in Figure 1. The remaining blank corn was mixed and packaged in 60 g aliquots into amber bottles. The final packaged units of SRM 1565 were irradiated to an absorbed dose of 11.9 kGy to 18.7 kGy by Neutron Products (Dickerson, MD).

Analytical Approach for Determination of Mycotoxins: Value assignment of the mass fractions of mycotoxins in SRM 1565 was based on the combination of measurements made by NIST using isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS), by FDA using ID-LC-MS/MS, and by collaborating laboratories, where available. Methods reported by collaborating laboratories are described in Appendix B.

NIST Analyses for Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2, Total Aflatoxins, Deoxynivalenol, Ochratoxin A, and Zearalenone Using ID-LC-MS/MS [1]: Mass fractions of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, total aflatoxins, and ochratoxin A were measured by ID-LC-MS/MS in duplicate 5.0 g test portions taken from each of 10 bottles of SRM 1565 using the internal standards listed in reference 2. Mass fractions of deoxynivalenol and zearalenone were measured by ID-LC-MS/MS in duplicate 1.0 g test portions taken from each of 10 bottles of SRM 1565 using the internal standards listed in reference 2. Each sample was hydrated with a volume of water and mycotoxins were extracted into 50:50 (volume fraction) water:acetonitrile using a large capacity mixer. Samples were centrifuged and filtered, and an aliquot of the supernatant was analyzed by positive-ion mode ID-LC-MS/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C18 column

were used for ID-LC-MS/MS determination of the mycotoxins. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the mycotoxins in the SRM following extraction. The same internal standard solutions containing stable isotope labeled mycotoxins were used for spiking the calibrants and samples. The calibration solutions used for value assignment of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, deoxynivalenol, and zearalenone were prepared using Certified Reference Materials (CRMs) from the European Commission Joint Research Centre (EC-JRC, Geel, Belgium). The calibration solutions used for value assignment of ochratoxin A were prepared using a CRM from the National Research Council Canada (NRCC, Ottawa, ON). The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in reference 3.

FDA Analyses for Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Deoxynivalenol, Fumonisin B_1, Fumonisin B_2, Fumonisin B_3, Total Fumonisins, T-2 Toxin, HT-2 Toxin, and Zearalenone Using ID-LC-MS/MS [2]: Mass fractions of aflatoxin B_1, aflatoxin B_2, and aflatoxin G_1 were measured by ID-LC-MS/MS in duplicate 5.0 g test portions taken from each of 10 bottles of SRM 1565 using the internal standards listed in reference 2. Mass fractions of deoxynivalenol, fumonisin B_1, fumonisin B_2, fumonisin B_3, total fumonisins, T-2 toxin, HT-2 toxin, and zearalenone were measured by ID-LC-MS/MS in duplicate 1.0 g test portions taken from each of 10 bottles of SRM 1565 using the internal standards listed in reference 2. Each sample was extracted into 50:50 (volume fraction) water:acetonitrile using a large capacity mixer. Samples were centrifuged and filtered, and an aliquot of the supernatant was analyzed by positive-ion mode ID-LC-MS/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C18 column were used for ID-LC-MS/MS determination of the mycotoxins. The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in reference 3.

Collaborating Laboratories' Analyses: Collaborating laboratories were asked to use their usual methods to make measurements on three test portions taken from one bottle each of SRM 1565 Mycotoxins in Corn (Blank) and SRM 1565 Mycotoxins in Corn (Incurred). Methods reported by collaborating laboratories are described in Appendix B.

Homogeneity Assessment: The homogeneity of mycotoxins in the SRM was assessed at NIST and FDA using the methods and test portion sizes described in this certificate (see "Instructions for Storage and Use"). Analysis of variance at a 5 % significance did not show statistically significant heterogeneity.

Coordination of the technical measurements leading to the certification of this SRM was performed by M.M. Phillips of the NIST Chemical Sciences Division. Analytical measurements at NIST were performed by T.M. López Seal and J.M. Ness of the NIST Chemical Sciences Division. Analytical measurements at FDA were performed by K. Zhang of the FDA/CFSAN. Analysts at many collaborating laboratories (Appendix A) analyzed SRM 1565 as part of an exercise of the NIST Health Assessment Measurements Quality Assurance Program (HAMQAP). Preparation, blending, and packaging of the SRM was conducted by A.J. Moors, J.M. Ness, J. Trevillian, and D.L. Ellisor of the NIST Chemical Sciences Division. Statistical analysis was provided by H.K. Liu of the NIST Statistical Engineering Division. Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

REFERENCES

[1] Phillips, M.M.; López Seal, T.M.; Ness, J.M.; Zhang, K.; *Development and Characterization of a Multi-Mycotoxin Reference Material*; J. AOAC Int. (2019) available at https://doi.org/10.5740/jaoacint.19-0109 (accessed July 2019).

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[7] Moors, A.J.; Pugh, R.S.; Evans, J.R.; Ness, J.M.; Ellisor, D.L.; Bachman, M.J.; *Standard Operation Procedures (SOPs) for the Palla VM-KT Vibrating Cryomill: Production of Fresh Frozen Standard Reference and Control Materials (SRMs/CMs) at the NIST Reference Material Production Facility*; NIST Special Publication 1196; National Institute of Standards and Technology, Gaithersburg, MD (2016); available at https://doi.org/10.6028/NIST.SP.1196 (accessed July 2019).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

NIST will monitor this SRM until its certification expires. If substantive technical changes occur that affect the certified values before this certificate expires, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. Contact the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, Maryland 20899-2300; telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

APPENDIX A

Collaborating Laboratories Contributing Data to Value Assignment of SRM 1565:

Academy of State Administration of Grain, China (Beijing, China)
Canadian Food Inspection Agency (Ottawa, ON, Canada)
Center for Analytical Chemistry, Department IFA-Tulln, BOKU Vienna (Tulln, Austria)
Dyad Labs (Salt Lake City, UT)
First Source Laboratory Solutions LLP (Hyderabad, TELANGANA, India)
HVL, LLC (Pittsburgh, PA)
International Food Safety Training Laboratory, Joint Institute for Food Safety and Applied Nutrition (JIFSAN) (College Park, MD)
ISURA (Burnaby, BC, Canada)
Laboratorio de Referencia de Alimentos y Aguas, Instituto Conmemorativo Gorgas (Panamá)
LACQSA/Lanagro-MG (Belo Horizonte, Minas Gerais, Brazil)
National Institute for Quality Control in Health, Oswaldo Cruz Foundation (INCQS, FIOCRUZ) (Rio de Janeiro, Brazil)
National Institute of Industrial Technology (INTI), Toxicology and Nutrition Laboratory (San Martín, Buenos Aires, Argentina)
National Metrology Institute of South Africa, Organic Analysis Section (Pretoria, South Africa)
National Referral Laboratory, ICAR-National Research Centre for Grapes (Pune, Maharashtra, India)
Natural Remedies (Bangalore, Karnataka, India)
Nature's Way (Green Bay, WI)
OMIC USA Inc. (Portland, OR)
SGS Canada Inc (Burnaby, BC, Canada)
Silliker JR Laboratories ULC (Burnaby, BC, Canada)
Taiwan Food and Drug Administration (Nangang, Taipei, Taiwan, Province of China)
Technological Laboratory of Uruguay (Montevideo, Uruguay)
US Food and Drug Administration, Office of Regulatory Affairs, Southeast Food and Feed Laboratory (SFFL) (Atlanta, GA)
US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science (College Park, MD)
US Department of Agriculture, Federal Grain Inspection Service, Technology and Science Division (Kansas City, MO)
Weck Laboratories, Inc. (City of Industry, CA)

APPENDIX B

Methods Reported by Collaborating Laboratories

Analyte(s)	**Method**
Aflatoxins	LC-MS/MS; LC-fluorescence; Enzyme-linked immunosorbent assay (ELISA)
Fumonisins	LC-MS/MS; LC-fluorescence
Deoxynivalenol	LC-MS/MS; LC-absorbance; Gas chromatography with electron capture detection (GC-ECD)
Zearalenone	LC-MS/MS; LC-fluorescence
Ochratoxin A	LC-MS/MS; LC-fluorescence
HT-2 Toxin	LC-MS/MS
T-2 Toxin	LC-MS/MS; LC-absorbance