



Utilization of a NIST SRM: a case study for per- and polyfluoroalkyl substances in NIST SRM 1957 organic contaminants in non-fortified human serum

Alix E. Rodowa¹ · Jessica L. Reiner¹

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Abstract

The National Institute of Standards and Technology (NIST) generates and maintains thousands of Standard Reference Materials (SRMs) to serve commerce worldwide. Many SRMs contain metrologically traceable mass fractions of known organic chemicals and are commercially available to aid the analytical chemistry community. One such material, NIST SRM 1957 Organic Contaminants in Non-Fortified Human Serum, was one of the first materials issued by NIST with measurements for per- and polyfluoroalkyl substances (PFAS) listed on the Certificate of Analysis and was commercially available in 2009. Since the release of SRM 1957, nearly 400 units have been sold to date, and over 50 publications related to PFAS measurements have included this material for multiple analytical purposes, such as a quality control material, for interlaboratory comparison, as an in-house comparison tool, for inter- and intra-day measurement accuracy, as an indicator of isomeric patterns of PFAS, and for other uses. This perspective details the ways SRM 1957 is utilized by the analytical community and how data have been reported in the literature. A discussion on accurately comparing SRM data to generated data is included. Furthermore, we conducted an in-depth investigation around additional applications for NIST SRMs, such as a matrix-matched reference material, and for the identification of targeted compounds during high-resolution mass spectrometry data collection. Ultimately, this manuscript illustratively describes the ways to utilize a NIST SRMs for chemicals of emerging concern.

Keywords PFAS · SRM · NIST · SRM 1957 · Reference material · Human serum

Introduction

The National Institute of Standards and Technology (NIST) produces a collection of Certified Reference Materials called Standard Reference Materials (SRMs). Production of new SRMs occurs every year, and existing SRMs are maintained and recertified as needed. NIST strives to support “accurate and compatible measurements by certifying and providing over 1300 Standard Reference Materials® with well-characterized composition or properties, or both [1].” Materials (SRMs) can contain either natural, endogenous contaminants (e.g., NIST SRM 1957 Organic Contaminants in

Non-Fortified Human Serum) or can be fortified (e.g., NIST SRM 1958 Organic Contaminants in Fortified Human Serum). Fortification of an SRM is designed to simulate contaminants in matrix, and can be fortified with any contaminants of interest, but are matrix relevant. NIST SRMs come with Certificates of Analysis, which contain details regarding the validated measurements, measures of uncertainty, and information about the maintenance and care of the SRM.

Measurements accompanied with an SRM have historically been delineated into “modes” which define the differences between certified values, reference values, and informational values [2]. However, a new modification to classifications has defined modes as certified values and non-certified values [3]. Certified values are generated by metrologically tracing contaminant mass fractions using a primary NIST-generated method (e.g., pyrolysis for purity and mass fraction-e.g., ng/g), and have values that are cross-validated with another method or methods with traceability back to the SI unit (e.g., by analysis using metrologically independent methods coupled

✉ Alix E. Rodowa
alix.robelt@nist.gov

¹ Chemical Sciences Division, National Institute of Standards and Technology, 331 Fort Johnson Rd., Charleston, SC 29412, USA

with SI-traceable calibrants). Non-certified values or reference values are reported as such because all sources of uncertainty have not been evaluated by NIST, but are comprised of measurements made from “the mean of samplings from one unit of material, the grand mean of results from multiple units of the material, or the median of an interlaboratory study [3].” Ultimately, NIST produces materials for commercial purchase in order to promote and support the needs of numerous sectors, and to promote and contribute to the production of high-quality measurements.

NIST SRM 1957 Organic Contaminants in Non-Fortified Human Serum

NIST SRM 1957 Organic Contaminants in Non-Fortified Serum was originally produced as a product for commercial sale on May 13, 2009, and has been maintained for over 10 years [4]. In total, SRM 1957 has certified values for seven polychlorinated biphenyl (PCB) congeners, three chlorinated pesticides, and five polybrominated diphenyl ethers (PBDEs), and has non-certified values, listed as reference values for 19 PCBs, seven per- and polyfluoroalkyl substances (PFAS), ten chlorinated dioxin and furan congeners, free and total cholesterol, phospholipids, triglycerides, and informational values (non-certified values) for PCB 180, tetrabromo- and chlorophenol, and Aroclor 1260 [4]. Development and production of the material resulted from a collaboration between NIST and US Centers for Disease Control and Prevention (CDC), and all reported contaminants occur without fortification in the pooled serum. The SRM Certificate of Analysis combines results from NIST and CDC methodologies which were determined by both gas chromatography-mass spectrometry (GC-MS) and/or liquid chromatography-mass spectrometry (LC-MS/MS) methods as well as interlaboratory studies [4]. Specifically, for PFAS, one NIST and one CDC method for the extraction and analysis by LC-MS/MS are included on the Certificate of Analysis. SRM 1957 contains reference mass fractions, or reference values, for perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) [4].

PFAS in SRMs

PFAS are ubiquitous, anthropogenic environmental contaminants [5–7], and are characterized by a fully or partially saturated C-F chain attached to a polar head group [8]. As a result of their physiochemical properties (e.g., hydrophobicity and oleophobicity), PFAS are used in many application including aqueous film-forming foams, manufacturing, and polymeric

coatings [9]. Because of proprietary and evolving PFAS manufacturing, thousands of PFAS have been released into the environment [10]. Two types of manufacturing exist, electrochemical fluorination which produces both branched and linear isomers of PFAS [11] and fluorotelomer manufacturing which produces primarily linear PFAS [12]. Two perfluoroalkyl substances, PFOA and PFOS, are the most well-known and studied PFAS, but other PFAS are of increasing interest to the public, military, and regulatory bodies. NIST SRMs containing PFAS can ensure increased measurement confidence and will be beneficial for serum and other matrices (e.g., SRMs for aqueous film-forming foams, groundwater, drinking water, and soil/sediment).

Ten NIST reference materials are available, containing mass fraction values for PFAS, including SRM 1946 Lake Superior Fish Tissue, SRM 1947 Lake Michigan Fish Tissue, SRM 1950 Metabolites in Frozen Human Plasma, SRM 1957 Organic Contaminants in Non-Fortified Human Serum (Freeze-Dried), SRM 1958 Organic Contaminants in Fortified Human Serum (Freeze-Dried), SRM 2585 Organic Contaminants in House Dust, SRM 2586 Trace Elements in Soil Containing Lead from Paint, SRM 2781 Domestic Sludge, RM 8446 Perfluorinated Carboxylic Acids and Perfluorooctane Sulfonamide in Methanol, and RM 8447 Perfluorinated Sulfonic Acids in Methanol [13].

Notably, PFAS are listed as non-certified values rather than certified values. The classification of PFAS as non-certified values was determined in 2009 as a result of the lack of high-purity, SI-traceable chemical standards for single isomeric species (e.g., linear PFOS). Since the original certification date, the National Metrology Institute of Japan now provides mass traceable, high-purity linear PFOA and linear PFOS (<https://unit.aist.go.jp/nmij/english/refmate/>). Several PFAS produced through ECF manufacturing co-occur in the environment as branched and linear isomers. Therefore, in order to generate separate certified measurements, values would need to be generated for each isomer of each PFAS. Each isomer would therefore have to be available commercially as a high-purity mixture, measured individually, and be traceable back to the SI unit. For PFOS, separate values would require six individual, SI-traceable branched isomers and one linear isomer. In addition, commercial analysis of PFAS by the United States Environmental Protection Agency (US EPA) [14, 15] requires mass fractions to be reported as a summation of all branched and linear isomers for PFHxS, PFOS, and other select ECF PFAS. Therefore, the reported NIST values are listed as total, summed branched and linear isomers of the ECF PFAS (e.g., total PFOS).

Utilizing current SRMs for PFAS analysis

Ten SRMs containing PFAS measurements are currently available for purchase, but there is little to no data reporting

the impact SRMs are having within the PFAS community. Given the large number of PFAS matrices represented, this study focused on one material, SRM 1957. The purpose of this study was to examine how the scientific PFAS measurement community is using SRM 1957. References for studies that reported the use of SRM 1957 were gathered using multiple literature databases. Of note, units of SRM 1957, comprised of 5 vials each, were purchased nearly 400 times over the last 10 years and were used for many purposes and contaminate classes. However, we chose to focus on the fraction of measurements made and reported for PFAS for clarity, and to explore the traditional analytical uses of SRM 1957 by the PFAS community, as well as new uses by the measurement community. In addition, we will discuss how to report experimental values, and the necessary comparison of the values on the Certificate of Analysis, as well as the underutilized applications that may be of broader interest.

Traditional uses of SRMs

As previously described by Wise et al. [2], certified reference materials, which are trade named SRMs at NIST, have traditionally been used for one of the following purposes: (1) to calibrate a measurement system, (2) to establish traceability of measurements, (3) to validate an analytical system, and (4) to provide quality control of measurements. Although calibration of an instrument is needed, calibration should not be applicable to SRM 1957 since it is a serum matrix and will therefore not be discussed further. Other solution reference materials, such as RMs 8446 and 8447, should be used for calibration purposes.

One of the main purposes of SRM 1957 is to validate an analytical system. Validation can be interpreted in several ways, including as a reference for analytical separation of branched and linear isomers, for confirmation of accuracy over time, and validation of an in-house control material. SRM 1957 was used by four separate studies, Riddell et al., 2009 [16], Martin et al., 2010 [17], Toms et al., 2019 [18], and Salihovic et al., 2013 [21] to validate analytical separation of branched and linear isomers of PFOS. These studies detail how SRMs can be used to determine the isomeric composition of PFAS in serum samples.

Validation of accuracy over time, within the same research group was also reported. In one example, Garcia et al., 2018 [19] used SRM 1957 to compare and validate inter-day variability to another study conducted in the same laboratory [20]. Another research group used SRM 1957 several times across many years, starting in 2013 [21], and being measured again in 2016 [22], 2018 [23], and 2020 [24]. Within each of the listed studies by Salihovic et al. [21, 23, 24] and Stubeleski et al. [22], the SRM was used extensively to control for inter-day variability. For example, Salihovic et al., 2013 [21] used the material 56 times over the course of 4 months. Not only is

the extensive material testing over time notable but demonstrates that the produced and published data is consistent and comparable over time.

Although the use of SRM 1957 was most prevalently presented to validate accuracy in a study, only a few studies used the material to validate an in-house reference material [24–27]. An SRM is purchased at a relatively high cost but can be used for validation of an in-house material which is produced by the laboratory at a lower cost. Once validated, an in-house material can be used for the duration of the study to ensure consistent quality data production with smaller or reduced consumption of the SRM and reduced overall cost. NIST encourages validation of in-house reference material since it promotes consistent accuracy of data quality over time. Furthermore, validation of an in-house reference material allows for economic savings since additional purchase of an SRM is unnecessary and is encouraged for long-term continuity and consistent data quality.

The most common use of SRM 1957 for PFAS analysis found in the literature is to provide quality control (QC) of measurements [28–39]. Similar to validation, QC of measurements can be interpreted in a number of different ways, for example, method and instrumental reproducibility (e.g., precision as % relative standard deviation (RSD), method and instrumental accuracy (e.g., % recovery) relative to the certificate of analysis values reported by NIST [4]. Of the 51 studies found, all of them (51/51) reported QC of measurement, but 33 of the 51 studies reported only QC of measurement for method and instrumental reproducibility. Eight of the 51 studies reported using the SRM for QC of measurement as part of an interlaboratory comparison (Table 1). Here, interlaboratory comparison was divided further to represent comparison as part of a laboratory validation study or as a comparison among labs to report a cohesive data set. A group of seven of the 51 total studies participated in an interlaboratory study and reported good agreement for QC of measurement [29, 41–43, 45, 46, 50]. An additional two studies, by Okada et al. [44] and Nakayama et al. [26], used SRM 1957 to aid as a means of comparing data among laboratories.

Reporting values and communicating comparison

SRMs have previously been used for quality control of measurements (Table 1). Importantly, a comparison of values documented in the NIST Certificate of Analysis and values produced by analyzing a NIST SRM through a laboratory methodology is the most effective way of establishing data quality. First, the values produced in a laboratory must report accuracy, typically as a mean value and a measure of uncertainty (e.g., standard deviation, standard error). For example, replicates of SRM 1957 may be extracted and analyzed as in Olsen et al., 2017 [51], with mass fractions reported as PFOS values of 20.5 ng/g, 19.6 ng/g, 20.9 ng/g, 20.0 ng/g, 19.1 ng/g,

Table 1 Description of SRM 1957 use where the study documented multiple uses. All other manuscripts only report QC of measurements

Publications	Validation of an analytical system			QC of measurements		PFAS identification
	Br/L separation*	Variability over time	In-house reference material	Method and instrumental reproducibility	Interlaboratory comparison	
Riddell et al., 2009 [16]	x			x		
Martin et al., 2010 [17]	x			x		
Toms et al., 2019 [18]	x			x		
Salihovic et al., 2013 [21]	x	x		x		
Stubleski et al., 2016 [22]		x		x		
Garcia, 2019 [40]		x		x		
Salihovic et al., 2020 [24]			x	x		
Lindstrom et al., 2009 [41]				x	x	
Lee & Mabury, 2011 [29]				x	x	x
Valsecchi et al., 2013 [42]				x	x	
Hanssen et al., 2013 [43]				x	x	
Okada et al., 2013 [44]				x	x	
Nøst et al., 2014 [45]				x	x	
Routti et al., 2016 [46]				x	x	
Nakayama et al., 2020 [26]			x	x	x	
Yeung et al., 2013 [47]				x		x
Gebbink et al., 2015 [48]				x		x
Gao et al., 2018 [49]				x		x

*Branched and linear isomer separation

20.8 ng/g, 19.6 ng/g, 19.0 ng/g, and 20.2 ng/g and which produced a mean value of 19.9 ng/g with a standard deviation of 0.62 ng/g (Fig. 1). The experimentally produced value was compared to NIST Certificate of Analysis values (e.g., PFOS is reported as 21.1 ng/g \pm 1.3 ng/g). As stated by the European Commission - Joint Research Centre Institute for Reference Materials and Measurements (IRMM), “comparison of values and uncertainty in which the difference in the mean values is less than the propagated uncertainty indicates that there is no significant difference between the measurement result and the certified value [52].” For the above example, the absolute difference between the average experimental value and certified value (19.9 ng/g–21.1 ng/g) is 1.2 ng/g, which is less than the error on the certificate of analysis value, 1.3 ng/g. Therefore, the value would indicate non-significant differences between the NIST reference value and the experimental value (an overlapping mean difference between the two measurements). The results of PFOS measurements in the literature [18, 25, 26, 28, 29, 32, 44, 51] can be compared to the values reported on the Certificate of Analysis for PFOS (Fig. 1). As shown in this figure, measurements made by the research groups typically fell within an acceptable range when compared to the values provided on the Certificate of Analysis. Alternatively, data could be compared by examining 95% confidence intervals.

Other applications of SRMs

Although there are many ways in which SRMs, and SRM 1957, have previously been used, several underutilized applications exist. Alternative applications include (1) use of an SRM as targeted compounds in matrix for high-resolution mass spectrometry (HRMS) data collection and (2) a matrix-matched material comprised of endogenous (non-fortified) chemicals and broaden the utility of SRMs for benchmarking measurements.

An emerging environmental data collection technique is that of HRMS. HRMS data can be collected as targeted data (e.g., the mass, identity, and retention time of the compound is known and can be quantified) or as non-target data (e.g., suspect analysis in which some information about the identity or structure may be known generally through a library match, or non-target analysis in which no identity or structural information is necessarily known). Since only targeted analytes are quantified in SRM 1957, targeted analytes can serve as sentinel compounds to frame other HRMS data. For instance, known compounds with known concentration or mass fractions and retention times can contribute to in-house HRMS libraries and as benchmark compounds elution times relative to other novel or less described analytes compounds.

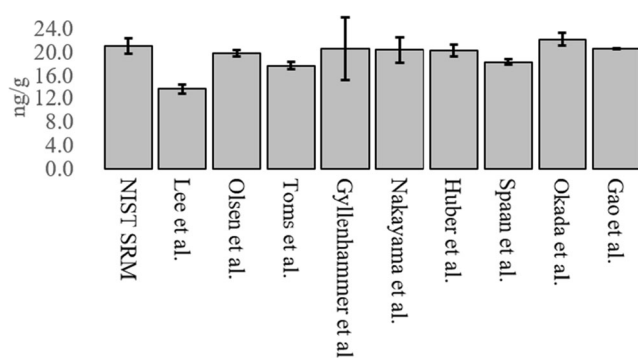


Fig. 1 Comparison of PFOS SRM 1957 Certificate of Analysis values [4] against experimental values [18, 25, 26, 28, 29, 32, 44, 51] with error reported in the original manuscript. Note that only publications which list values and errors for total PFOS were included in the figure

In a few studies, by Lee and Mabury [29], Yeung et al. [47], Gao et al. [49], and Gebbink et al. [48] additional compounds were identified in SRM 1957 which were not previously described in the Certificate of Analysis. These analytes included N-ethylperfluorooctanesulfonamid (N-EtFOSA), 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA), perfluorobutanoic acid (PFBA) and perfluorobutane sulfonate (PFBS) [49], perfluorodecane sulfonate (PFDS), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorooctanesulfonamide (FOSA), perfluorooctanesulfonamidoacetic acid (FOSAA), N-methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA), N-ethylperfluorooctanesulfonamidoacetic acid (EtFOSAA), and a series of n:2 perfluoroalkyl phosphate diesters (diPAPs) [29, 47–49]. It is therefore likely that other compounds will be discovered during HRMS data collection for SRM 1957. Since the values for the above compounds were not determined by NIST, they are not included on the Certificate of Analysis. However, publishing results for newly observed PFAS is beneficial to the greater scientific community and could inform future measurements made by NIST.

SRMs can be utilized as a representative matrix material. For instance, SRM 1957 has been used to verify and validate branched and linear isomeric composition of PFOS in other serum samples. Additionally, commercially available standards, which have branched and linear isomeric components, are currently limited to the more commonly observed analytes including PFHxS, PFOS, N-EtFOSAA, and N-MeFOSAA. Therefore, the use of a matrix-matched material which contains non-fortified PFAS isomeric composition is an excellent means of validating separation of branched and linear components and also for qualitative identification of various isomers for in-house (HRMS) libraries (e.g., PFHpA). This is especially helpful when retention times and ionization efficiency information for branched isomers are not present in analytical standards with only linear isomers. For example, in Riddell et al., the difference in ionization efficiency of each isomer is described [16]. To accurately report total branched and linear

composition, branched and linear isomers should be integrated and included in the total concentration or mass fraction. As PFAS determination in blood becomes more common with the addition of new PFAS compounds, matrix-matched materials will be more necessary for data quality and validation.

With interest from stakeholders increasing about PFAS, the use of SRMs will be imperative for increased data quality around PFAS measurements. As described, SRMs can and should be used for traditional uses, but should also be used for new and creative purposes. The accuracy of data measurements should be conducted with care but can aid in reporting accuracy (QC of measurement), method validation, and control of measurements. In the 10 years since the release of SRM 1957, over 50 publications used and reported the SRM for PFAS measurements, although many other publications exist for other contaminant measurements. The large use and reporting of PFAS values indicate the need for SRMs.

Code availability Not applicable.

Author contribution Alix Rodowa—primary author. Jessica Reiner—editing, contributions to validity and accuracy.

Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent for publication The authors provide consent for publication of this manuscript.

Conflict of interest The authors declare no competing interests.

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Alix Rodowa is an NRC postdoctoral researcher at the National Institute of Standards and Technology in Charleston, SC. She has been working on method development, analytical measurements, and reference materials for per- and polyfluoroalkyl substances in various matrices.



Jessica Reiner is a research chemist at the National Institute of Standards and Technology in Charleston, SC, and program coordinator in the Biochemical and Exposure Science Group. She has been working for several years on improving analytical measurements of per- and polyfluoroalkyl substances (PFAS) through the certification of reference materials, methods development, and other quality assurance activities.