NIST

Date of Issue:
27 April 2018

Standard Reference Material® 968f
Fat-Soluble Vitamins in Frozen Human Serum
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended for use in validating methods for determining fat-soluble vitamins in human serum and plasma and qualifying control materials produced in-house and analyzed using those methods.

Description: A unit of SRM 968f consists of one vial each of two concentration levels of frozen human serum, each vial containing at least 1.05 mL serum.

Certified Values: Certified values are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias have been accounted for.

Table 1. Certified Values for Fat-Soluble Vitamins in SRM 968f
Values are metrologically traceable to the International System of Units (SI) through the molar absorptivities of the calibration standards.

	Level 1[a]		Level 2[a]	
Analyte	µg/mL	µmol/L[b]	µg/mL	µmol/L[b]
Total Retinol[c]	0.327 ± 0.013	1.141 ± 0.045	0.658 ± 0.028	2.30 ± 0.10
α-Tocopherol	5.15 ± 0.21	11.95 ± 0.49	11.85 ± 0.73	27.5 ± 1.7
γ+β-Tocopherol[d]	1.094 ± 0.049	2.63 ± 0.12	2.59 ± 0.14	6.21 ± 0.34

[a] Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

[b] Amount concentration values, mol/L, are calculated from the mass concentration results, microgram per milliliter, via multiplication by 1000/M, where M is the molar mass, grams per mol, of the analyte. The molar masses used are: M_{retinol} = 286.5 g/mol, $M_{\alpha\text{-Tocopherol}}$ = 430.7 g/mol, and $M_{\gamma+\beta\text{-Tocopherol}}$ = 416.7 g/mol. These molar masses have associated standard uncertainty $u(M)$ = 0.05 g/mol.

[c] Total Retinol includes *cis*- and *trans*-retinol.

[d] γ+β-Tocopherol includes γ- and β-tocopherol. These analytes coelute in most chromatographic systems.

Expiration of Certification: The certification of **SRM 968f** is valid, within the stated measurement uncertainty, until **31 December 2025**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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Certificate Revision History on Last Page

Non-Certified Values: Tables 2 through 4 list values that do not meet NIST's criteria for certification but are the best currently available estimates for measurands of potential interest. Non-certified values, formerly known as NIST Reference and Information Values, may include consensus values and results from method-specific protocols and collaborating laboratories.

Table 2. Consensus Values for Selected Vitamin-Related Analytes in SRM 968f[a]

These non-certified values are metrologically traceable to calibration procedures and standards used within this experienced measurement community.

Analyte	Units	Level 1 $x \pm U_{95\%}(x)$[b]	Level 2 $x \pm U_{95\%}(x)$[b]	M[c] g/mol
Total β-Carotene[d]	µg/mL	0.111 ± 0.011	0.195 ± 0.021	536.9
Trans-β-Carotene	µg/mL	0.098 ± 0.021	0.174 ± 0.039	536.9
Cis-β-Carotene	µg/mL	0.011 ± 0.010	0.015 ± 0.011	536.9
Total α-Carotene[d]	µg/mL	0.026 ± 0.007	0.012 ± 0.012	536.9
Total Lycopene[d]	µg/mL	0.154 ± 0.028	0.58 ± 0.12	536.9
Trans-Lycopene	µg/mL	0.084 ± 0.015	0.293 ± 0.047	536.9
Total β-Cryptoxanthin[d]	µg/mL	0.030 ± 0.008	0.044 ± 0.017	552.9
Total Lutein[d]	µg/mL	0.036 ± 0.010	0.087 ± 0.037	568.9
Total Zeaxanthin[d]	µg/mL	<0.05[e]	0.029 ± 0.021	568.9
Total Lutein+Zeaxanthin[f]	µg/mL	0.052 ± 0.006	0.115 ± 0.019	568.9
Retinyl Palmitate	µg/mL	0.017 ± 0.015	0.030 ± 0.029	524.8
Coenzyme Q10	µg/mL	0.500 ± 0.034	1.21 ± 0.11	863.3
Phylloquinone (K_1)	ng/mL	0.227 ± 0.047	0.69 ± 0.14	450.7
25-Hydroxyvitamin D	ng/mL	14.0 ± 4.6	16.9 ± 4.0	400.7

[a] Analytes reported by at least four participants for these materials in the 2016 Summer, 2017 Winter, and 2017 Summer MMQAP studies.

[b] Values are expressed as $x \pm U_{95\%}(x)$, where x is the consensus value and $U_{95\%}(x)$ is the expanded uncertainty of the consensus value. The true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

[c] Molar mass of the analyte with associated standard uncertainty u(M) = 0.05 g/mol. The amount concentration corresponding to a value x of the mass concentration (expressed in grams per milliliter) is 1000x/M (expressed in mol per liter).

[d] "Total" indicates that the value reported is the sum of *trans*- and *cis*-isomers of the analyte.

[e] Due to a combination of a small number of reported results and disagreement between those results, this value represents the upper bound of a 95 % level of confidence interval with a lower bound of 0.

[f] The sum of the mass concentrations of lutein and zeaxanthin isomers. These analytes coelute in many chromatographic systems.

Table 3. Method-Specific Vitamin D Metabolite Mass Fractions in SRM 968f[a]

These non-certified values are metrologically traceable to the measurement methods and the calibration procedures and standards used.

Analyte	Units[b]	Level 1		Level 2		M[e]
		n[c]	$x \pm U_{95\%}(x)$[d]	n[c]	$x \pm U_{95\%}(x)$[d]	g/mol
25-Hydroxyvitamin D_2[b]	ng/g	4	0.834 ± 0.050	4	0.164 ± 0.014	412.7
25-Hydroxyvitamin D_3[b]	ng/g	6	12.10 ± 0.20	6	15.33 ± 0.21	400.7
3-*epi*-25-Hydroxyvitamin D_3[b]	ng/g	6	0.707 ± 0.033	6	1.05 ± 0.14	400.7

[a] Determined at NIST using isotope-dilution liquid chromatography-tandem mass spectrometry as described in SP 260-188.

[b] To convert from mass fraction (microgram per gram) to mass concentration (microgram per milliliter), multiply $x \pm U_{95\%}(x)$ by the density listed in Table 4.

[c] Number of independent measurements.

[d] 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 method-specific true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, model the measured value of the mass concentration as a random variable of the form $x + \sigma T$, where T denotes a Student's t random variable having $n - 1$ degrees of freedom with $\sigma = (U_{95\%}(x)/t_{0.975,n-1})\sqrt{(n-3)/(n-1)}$. Defined in this manner, the random variable $x + \sigma T$ has mean x and standard deviation $u(x)$.

[e] Molar mass of the analyte with associated standard uncertainty $u(M) = 0.05$ g/mol. The amount concentration corresponding to a value x of the mass concentration (expressed in micrograms per gram) is $1000\rho x/M$ (expressed in mol per liter), where ρ denotes the serum mass density listed in Table 4.

Table 4. Method-Specific Serum Densities of SRM 968f[a] at 22.6 °C

These non-certified values are metrologically traceable to the measurement method and the calibration procedures and standards used.

Measurand	Units	Level 1[b]	Level 2[b]
Serum Density at (22.6 ± 0.2) °C[c]	g/mL	1.0180 ± 0.0004	1.0202 ± 0.0004

[a] Determined at NIST as described in SP 260-188.

[b] Values are expressed as $x \pm 2s$, where x is the measured value and s is the standard deviation of the method. The method-specific true value of the measurand is believed to lie within the interval $x \pm 2s$ with 95 % confidence. To propagate this uncertainty, treat the value as a normally distributed random variable with mean x and standard deviation s.

[c] Measurements made at ambient temperature in a temperature-controlled balance room. The stated uncertainty is a conservative estimate of the 95 % expanded uncertainty.

Storage and Handling: Until required for use, SRM 968f should be stored in the dark at or below −70 °C. If total retinol and the tocopherols are the only analytes of interest, then SRM 968f may be stored in the dark at −20 °C.

Use: SRM 968f is provided as a set of two vials of frozen serum that should be allowed to thaw at room temperature for at least 30 min under subdued light. The contents of a vial should then be gently mixed prior to removal of a test portion for analysis. Precautions should be taken to avoid exposure to strong ultraviolet (UV) light and direct sunlight. The certification only applies to the initial use and the same results are not guaranteed if the remaining material is used at a later date.

Safety: This is a human source material. Handle product as a biohazardous material capable of transmitting infectious disease. The supplier has reported that each donor unit of plasma used in the preparation of this product was tested by FDA-licensed tests and found to be negative for human immunodeficiency virus (HIV), HIV 1 antigen, hepatitis B surface antigen, and hepatitis C. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2.

Source: This SRM was developed after an appropriate human subjects research determination by NIST.

Additional Information: Full details on the production, analysis, and statistical evaluation of SRM 968f are provided in: NIST Special Publication 260-188, *Certification of Standard Reference Material® 968f Fat-Soluble Vitamins in Frozen Human Serum.* This publication is available free of charge at https://doi.org/10.6028/NIST.SP.260-188.

Certificate Revision History: 27 April 2018 (Deletion of the word "Total" from the analyte description for cis-β-carotene in Table 2.; editorial changes); 14 December 2017 (Original certificate issue date).

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.