



September 14, 2017

Ms. Tammy Blake, Quality Manager
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601 E. Jackson Street
Richmond, Virginia 23219

RE: Measurement Uncertainty Data for Smokeless Tobacco Methods

Altria Client Services estimates measurement uncertainty using ASTM Method E2554-13 "Standard Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method using Control Chart Technique". This practice describes techniques for a laboratory to estimate "Type A" uncertainty of a test result using data from test results on a control sample. "Type B" uncertainty has been included using stated uncertainty from Certificate of Analysis of standard reference materials, where applicable. Laboratories accredited under ISO/IEC 17025 are required to present uncertainty estimates for their test results. This practice provides procedures that use test results to develop uncertainty estimates for an individual laboratory.

- Type A Uncertainty estimates are obtained as the standard deviations of repeated measurement results, such as repeated measurements of a homogenous control sample that is taken through all the steps of a method and analyzed throughout an analytical run.
- Type B Uncertainty estimates are obtained by other means than the statistical treatment of repeated measurement results. The other means can be certificates of reference materials, specifications, manuals of instruments, estimates based on long-term experience, etc.

Altria Client Services is accredited to ISO/IEC 17025 Standard by the American Association for Laboratory Accreditation (A2LA), Cert. No. 0660.01. The Scope of Accreditation can be located on the A2LA web site.

- [Table I](#) is a list of methods for testing tobacco that are within Altria's scope of accreditation.
- [Table II](#) describes the estimated measurement uncertainty for these method showing Type A, Type B, and expanded combined uncertainties (k=2) using ASTM E2554 – 13.

Sincerely,

David Self
Contractor, DCR Workforce
Altria Client Services

Table I Test Methods

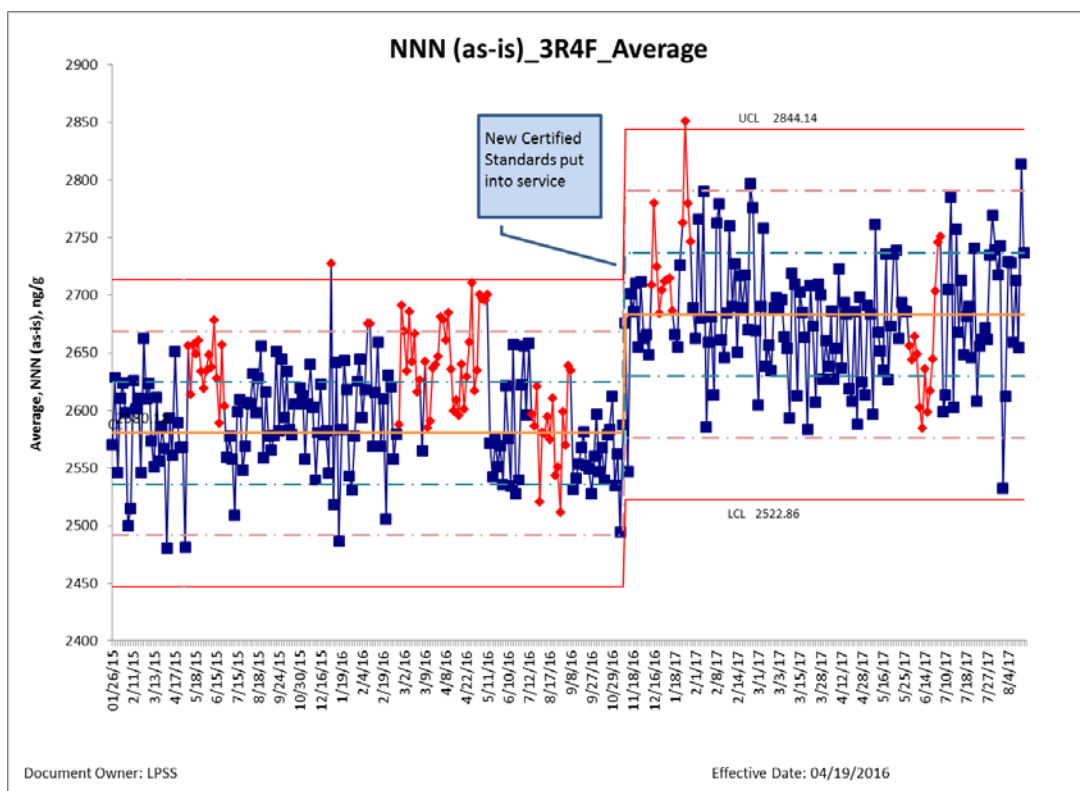
Test Number	Test Description	Referenced Methods
095-5519	Determination of Tobacco Specific N-Nitrosamines in Tobacco and Tobacco Products by LC-MS/MS	CORESTA Recommended Method N°72
095-5021	Determination of Benzo[a]Pyrene in Tobacco Products by GC-MS	
095-5020	Determination of Select Carbonyls in Tobacco by UPLC / MS /MS	
095-5529	Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatography	FR Vol. 74, No. 4, 01/07/2009 pp. 712-719
095-5507	Analysis of Cadmium and Arsenic in Tobacco Products by ICP-MS	
095-3370	Determination of pH by the Center for Disease Control Method	FR Vol. 74, No. 4, 01/07/2009 pp. 712-719
095-3371	Determination of Total Moisture by the Center for Disease Control Method	FR Vol. 74, No. 4, 01/07/2009 pp. 712-719

Table II – Estimated Measurement Uncertainties

Measurement Uncertainty as Percent based on 7 replicates				Measurement Uncertainty Example			
Analyte	Type A %	Type B %	Combined Expanded %	Example Level	Std. Dev.	Expanded Uncertainty	Units
NNN	2.5	0.5	3.8	2679.5	43.9	102	ng/g
NNK	3.5	0.5	3.6	772.5	22.1	27.8	ng/g
B[a]P	6.5	1.3	10.1	8.4	0.3	0.85	ng/g
Formaldehyde	10.6	2.5	19.2	6.2	0.57	1.2	µg/g
Acetaldehyde	10.3	2.5	17.8	2.9	0.39	0.52	µg/g
Nicotine	1.6	1.3	4.0	1.04	0.005	0.04	%
Nicotine (low)	5.5	1.3	8.9	0.73	0.016	0.06	%
Arsenic	3.5	0.25	4.9	206.4	5.0	10.1	ng/g
Cadmium	3.1	0.25	5.0	1143.6	17.2	57.2	ng/g
pH	(b) (4)						pH
Moisture							%

Control Charts:

ALCS uses Average and Range control charts to monitor method performance. Limits are set in which out-of-control data is outside of 3-sigma from the mean and trends are identified if more than 8-points in-a-row on one side of the mean (points in red). ALCS will repeat analytical runs in which monitor data is outside of 3-sigma with notation on the log sheet. Results are closely monitored when 8-points in a row are on one side of the mean. If trending results are not corrected within a specific time frame, (repeated points outside 3-sigma or points in-a-row on one side of the mean, root cause analysis is performed to determine an assignable cause. Below is the control chart for N-nitrosornnicotine (NNN); there was a mean shift when switching to new certified reference standards, there are a few data points outside of 3-sigma which resulted in batch repeats and there were some trends of more than 8-points on one side of the mean that were corrected.



Calculating measurement uncertainty utilizes all acceptable data from the control chart. For NNN, 3 replicates are performed using (b) (4).

Statistical Method:

1. An excel workbook is utilized to calculate measurement uncertainty using valid monitor data, calculating the Average and Standard Deviation of the 2 or more replicates. The Standard Deviation (StdDev) is squared and the sum of all the standard deviations squared (s^2) is used to calculate s_r -- the estimate of a single laboratory repeatability standard deviation.

a. $\sum s^2 = \text{Sum of (StdDev)}^2$ where $p = \text{number of runs}$

$$s_r = \sqrt{\frac{\sum s^2}{p}}$$

- b. Below is a screen shot of the NNN data for (b) (4) from 11/2016 to 7/2017. Several rows of data are hidden to due to space limitations. In this case, there were ~125 data values used to estimate measurement uncertainty.

SOP 095-5519 -- Determination of TSNA in Tobacco and Tobacco Products by LC-MS/MS						
Uncertainty Calculation NNN using (b) (4) as the Monitor						
Date of Entry	Rep #1 ng/g	Rep #2 ng/g	Rep #3 ng/g	Average	StdDev	SD^2
11/14/16	2732.60	2635.00	2652.00	2673.20	52.14	2718.5
11/15/16	2566.10	2569.30	2506.00	2547.13	35.66	1271.5
11/17/16	2723.30	2716.80	2662.80	2700.97	33.21	1103.1
11/18/16	2691.80	2683.70	2682.30	2685.93	5.13	26.3
11/28/16	2692.10	2694.70	2744.20	2710.33	29.36	861.9
11/29/16	2664.30	2627.70	2672.70	2654.90	23.93	572.5
12/1/16	2705.00	2707.40	2722.40	2711.60	9.43	88.9
12/6/16	2654.20	2711.60	2622.40	2662.73	45.21	2043.8
7/5/17	2632.88	2552.95	2610.87	2598.90	41.29	1704.7
7/10/17	2553.30	2632.10	2654.50	2613.30	53.15	2825.4
7/10/17	2716.45	2696.79	2700.97	2704.74	10.36	107.3
7/12/17	2865.50	2746.40	2743.80	2785.23	69.53	4833.7
7/12/17	2677.70	2584.20	2545.78	2602.56	67.85	4603.5

2. Since there is a between sample and between time variation, an estimate of the between time standard deviation is computed (s_{time}). First, the standard deviation among the sample averages is determined and squared, (s_x^2), where s_x is standard deviation of all the average results. Using the equation below, s_{time} is determined.

S_x = standard deviation of the averages, S_r = single laboratory repeatability, n = number of replicates per run

$$s_{\text{time}} = \sqrt{s_x^2 - \frac{s_r^2}{n_{\text{within}}}}$$

3. The Uncertainty standard deviation is estimated from a single time and a single repeat using the equation below. This value is equivalent to an estimate of intermediate precision based on multiple time periods and is called Type A combined. S_r^2 is divided by the number of replicates where n_{within} is the number of replicates of the monitor (usually 2 or 3) and n_{reps} is the number of replicates for a typical smokeless tobacco sample (usually $n=7$).

S_u = Type A Combined -- see chart below

$s_r = \sqrt{\frac{\sum s^2}{p}}$	NNN - s_{rLT} (long term within run)		49.37224
$s_{time} = \sqrt{s_x^2 - \frac{s_r^2}{n_{within}}}$	NNN - s_{time} (ng/g, Time and Run)	46.0	
$s_u = \sqrt{s_{time}^2 + \frac{s_r^2}{n_{within}}}$	NNN - Monitor - Type A Combined Uncertainty (n=3)	54.1	2.0%
$s_u = \sqrt{s_{time}^2 + \frac{s_r^2}{n_{reps}}}$	NNN - Product Type A Combined Uncertainty (n=7)	49.6	1.9%

4. The total uncertainty (U_C) is calculated using both Type A ($s_u = U_A$) uncertainty and Type B uncertainty (U_B) obtained from the Certificate of Analysis of the NNN Certified Reference Standard. The Expanded Uncertainty at 95% Confidence Interval is U_C multiplied by the k Factor, which in two.

$$U_C = \sqrt{U_A^2 + U_B^2}$$

$$U_{95} = U_C \times \text{"k" Factor}$$

5. The total uncertainty for NNN is shown below as a percentage.

Calculating Combined A and B Uncertainties		
U_C = Combined Uncertainty (n=3)		2.1%
U₉₅ = Expanded Uncertainty @ 95% Confidence Level	U₉₅ = U_C X "k" Factor	4.2%
"k" factor = 2.0		
U_C = Combined Uncertainty (n=7)		1.9%
U₉₅ = Expanded Uncertainty @ 95% Confidence Level	U₉₅ = U_C X "k" Factor	3.8%

6. Below is an example of a report of the average, standard deviation and the estimated expanded uncertainty for NNN ground (b) (4) monitor results when performing three replicates and seven replicates.

NNN - (b) (4)	Average	2679.5	ng/g
	Standard Deviation (3 reps)	43.9	ng/g
U = Expanded Uncertainty (k=2) (n=3, 4.2%)	U @95% two-sided Confidence Intervals	112.5	ng/g
U = Expanded Uncertainty (k=2) (n=7, 3.8%)	U @95% two-sided Confidence Intervals	101.8	ng/g