College of Agriculture, Food and Environment
Center for Tobacco Reference Products

University of Kentucky
Center for Tobacco Reference Products

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SMK-2022D Proficiency Program Protocol

2022 Round 4

Objective

This round of testing will include analysis of chemical constituents within two certified reference smokeless tobacco products: 1S4 (Swedish Style Snus) and 3S3 (Moist Snuff). ***Please do not round results at any point in your calculations.*** **Treat the proficiency testing material in the same manner as the majority of routinely tested samples.**

The chemical constituents are:

- Total Nicotine
- Free Nicotine
- NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
- NNN (N-nitrosonornicotine)
- NAT (N-nitrosoanatabine)
- NAB (N-nitrosoanabasine)
- Acetaldehyde
- Crotonaldehyde
- Formaldehyde
- Benzo[α]pyrene
- Cadmium
- Arsenic
- pH
- Moisture

Proficiency Study Timeframe

August 25, 2022: 09:00 AM EDT	*PT round Opens, Test Kits available for purchase*
October 27, 2022: 09:00 AM EDT	*Data submission portal Opens, First day of data submission*
January 05, 2023: 5:00 PM EST	*Data submission portal Closes, Final day of data submission*
January 26, 2023	*Target date for issuance of Interim Report*
February 23, 2023	*Target date for issuance of Final Report, PT round Closes*

Eastern Daylight Time (EDT) (New York, NY time)
Eastern Standard Time (EST) (New York, NY time)

Please check ctrp.uky.edu for updates during the study. To request an extension, please contact ruth.mcnees@uky.edu or ctrp@uky.edu.

Test kits are available for purchase beginning on August 25, 2022. This round of testing for data submission will open on October 27, 2022 and close on January 5, 2023. The University of Kentucky, Center for Tobacco Reference Products (CTRP) data submission portal will be locked after the closing date and will no longer accept results. Results obtained after the closing date will not be included in the proficiency study report. The target date for issuance of the interim report is January 26, 2023. The participants are encouraged to review the interim report and provide feedback, i.e. comments, erroneous data entry, additional notes, etc., through the online feedback form available by clicking "Submit Comments" next to the interim report link located on the "My Proficiency Studies" tab of the CTRP website (ctrp.uky.edu). Feedback received will be considered and, if necessary, incorporated in a final report which will tentatively be issued on February 23, 2023. The interim report and final report can be downloaded from the "My Proficiency Studies" tab located on the CTRP website by clicking the "Interim Report" or "Analysis Report" link, respectively.

References

Conditioning	
Nicotine	CORESTA N 87, CORESTA N 62
NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	CORESTA N 72, ISO/TS 22304:2008
NNN (N-nitrosonornicotine)	CORESTA N 72, ISO/TS 22304:2008
NAT (N-nitrosoanatabine)	CORESTA N 72, ISO/TS 22304:2008
NAB (N-nitrosoanabasine)	CORESTA N 72, ISO/TS 22304:2008
Acetaldehyde	CORESTA N 86
Crotonaldehyde	CORESTA N 86
Formaldehyde	CORESTA N 86
Benzo[α]pyrene	CORESTA N 91
Cadmium	CORESTA N 93
Arsenic	CORESTA N 93
pH	CORESTA N 69
Moisture	CORESTA N 76

Note: Not all chemical constituents have a reference

Proficiency Test Materials include 1S4 (Swedish Style Snus) and 3S3 (Moist Snuff)

Proficiency Test Materials for this round of proficiency testing must be obtained from the CTRP by procuring the proficiency test kit. The materials will come with a test protocol and instructions to download the electronic reporting template in the form of a pre-formatted MS Excel file. It is not acceptable to use 1S4 (Swedish Style Snus) or 3S3 (Moist Snuff) from your inventory. Homogeneity of the Proficiency Test Material was determined by sub-contracting analysis to four third-party laboratories meeting the quality requirements of the proficiency testing scheme in accordance with ISO/IEC 17043. Test results confirm that the Proficiency Test Material is fit for proficiency testing. Stability testing is on-going and accumulated data show mean values to be stable within ±15% of the reference value from the Certificate of Analysis for the certified reference smokeless tobacco products available at the CTRP website (ctrp.uky.edu).

General Guidance

It is important to note the need for participants to record any deviation from the standard methods in their report. Operating conditions considered optional reporting by the laboratory should also be recorded on their report. Any circumstances that arise during the analysis of these products which may influence either the precision or the bias of the result should be recorded in the report. Details of deviation from normal operations should be recorded in the "Notes" section of the Excel reporting template.

Based on feedback from our proficiency testing participants, we anticipate participation from approximately 10 laboratories for this Proficiency Testing Scheme. Any participant that does not receive a proficiency testing kit or receives a damaged kit is encouraged to contact the CTRP (ctrp@uky.edu) immediately to ensure that the participant has sufficient time to complete the Proficiency Test Scheme as scheduled.

Test Item Storage

The samples should be stored in plastic bags at -20°C prior to preparing for the proficiency test.

Handling Smokeless Tobacco Products

The samples shall be moved to refrigerated conditions (4°C) 24 hours prior to use and equilibrated to room temperature for at least 2 hours before use. After this initial equilibration, the samples shall be stored in a refrigerator for up to one week in between use. Samples cannot be refrozen and stored for future proficiency testing.

The Snus pouches should be cut into 2 halves directly into the extraction vessel. Both Snus and paper are to be analyzed.

Replicates Required

Replicates must be obtained **under repeatability conditions** i.e. same instrument and same operator**.** Analysis of 5 replicates for each of the two smokeless tobacco products should be performed. Laboratories should follow their routine analytical plan.

Participants who order a Proficiency Test Kit should download the Excel reporting template which will be used to submit results for the proficiency testing. The Excel reporting template can be downloaded from the "My Proficiency Studies" tab located on the CTRP website (ctrp.uky.edu) after you have purchased a Proficiency Test Kit. If you order multiple kits, you must download the Excel reporting template for each of the Proficiency Test Kits. Each Excel reporting template has a unique "Assigned Data Set ID" based on a customer's purchase. Please make sure that you enter the data into the correct Excel reporting template. *Please do not round results at any point in your calculations. Make sure to report results in the units indicated in the Excel reporting template. Please note that the requested minimum significant digits are set forth in the CORESTA Recommended Methods identified as references for each parameter.* The results should be submitted electronically through the CTRP website on the "My Proficiency Studies" tab. The participant will: (1) click the blue "Submit Proficiency Data" button for the correct reporting proficiency study; (2) browse their computer for the Excel reporting template for that proficiency study; (3) select the appropriate file; and (4) then click the "Load and Review Data"

button. The participant will have the opportunity to review their data online before their final submission of data to the CTRP.

The study report will contain:

- Executive summary
- Purpose of study
- Protocol
- Coded laboratory raw data
- Statistical summary and ***z-***score by laboratory (both graphical and numeric)

File Formatting Requirements for Data

To ensure clear and uninterrupted data processing among disparate computer systems, please use the Excel reporting template provided with the Proficiency Test Kit, which has been formatted for data entry. Please note that the downloadable Excel reporting template contains "locked" codes and a Proficiency Study ID (SMK-2022D) and an Assigned Data Set ID number specific to your test kit and this round of Proficiency Testing.

Common sources of data error include, but are not limited to, incorrect units for reporting data, failure to calculate values for individual smokeless products, or improper calibration.

Below is a description of the file formatting, type, and expected contents of data files to be sent to the CTRP Proficiency Testing Program.

File Details

The data transport file should be formatted as an Excel file, specifically the XML-based format that Excel files are saved in by default. There should be no spaces in the filename. The Excel file extension should, by default, be *.xlsx.*

example: *smokeless_datasetid_3476.xlsx*

Proficiency Data

Please use the dropdown box in the top right section of the Excel reporting template to answer whether the lab has ISO Accreditation.

Data

Please be sure to enter measurements for the specific Data:

- *Test Date for each Smokeless Product.*
- *Measurements for each of the 14 chemical constituents (Total and Free Nicotine, NNK, NNN, NAT, NAB, Acetaldehyde, Crotonaldehyde, Formaldehyde, Benzo[α]pyrene (BaP), Cadmium, Arsenic, pH, and Moisture) for each of the 5 replicates.*

- *Please use the dropdown menu to select the "method" used in testing for each of the 4 TSNA chemical constituents. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	NNK	NNN	NAT	NAB
Method 1	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS
Method 2	GC/TEA	GC/TEA	GC/TEA	GC/TEA
Enter alternate method (text)				

For the instrument:
LC/MS/MS: Liquid Chromatography-Tandem Mass Spectrometry
GC/TEA: Gas Chromatography-Thermal Energy Analyzer

- *Please use the dropdown menu to select the "internal standard" used in testing for each of the 4 TSNA chemical constituents. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	NNK	NNN	NAT	NAB
Internal Standard Option 1	NNK-d4	NNN-d4	NAT-d4	NAB-d4
Internal Standard Option 2	NDHA	NDHA	NDHA	NDHA
Internal Standard Option 3	NNPA	NNPA	NNPA	NNPA
Other (enter text)				

NNPA: N-Nitrosopentyl-(3-picolyl)-amine; NDHA: N-hexyl-N-nitroso-1-hexanamine

- *Please use the dropdown menu to select the "method" used in testing for each of the 6 chemical constituents. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	acetaldehyde	crotonaldehyde	formaldehyde	BaP	As	Cd
Method 1	UHPLC-MS/MS	UHPLC-MS/MS	UHPLC-MS/MS	GC/MS	ICP-MS	ICP-MS
Method 2	GC-MS	GC-MS	GC-MS	LC/FLD	ICP-OES	ICP-OES
Method 3					AAS	AAS
Enter alternate method						

For the instrument:
UHPLC- MS/MS – Ultra High Performance Liquid Chromatography- Mass Spectrometry/ Mass Spectrometry
GC-MS- Gas Chromatography-Mass Spectrometry
LC/FLD: Liquid Chromatography-Fluorescence Detector
ICP-MS – Inductively Coupled Plasma Mass Spectrometry
ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy
AAS: Atomic Absorption Spectroscopy

- *Please use the dropdown menu to select the "internal standard" used in testing for each of the 4 chemical constituents. If your standard is not identified in the dropdown menu, please type your method in the box provided.*

	acetaldehyde	crotonaldehyde	formaldehyde	BaP
Internal Standard	Acetaldehyde-d4	Crotonaldehyde-d3	Formaldehyde-d2	BaP-d12
Other				

- If a participant does not have a measurement for a data field, please leave the Excel cell blank. When you upload your spreadsheet to the CTRP database, you will have the opportunity to review all your data on the web-based user interface. All cells that were left blank on the Excel reporting template, will appear as "< empty >" on the web-based user interface screen.

File Data

Additional information/instructions are available on the home page of the CTRP website in the document section at How to Upload Proficiency Test Data (OR https://ctrp.uky.edu/assets/pdf/webdocs/How_to_Upload_Proficiency_Test_Data.pdf).

Completed Files

When the Excel reporting template is completed and saved with the current date embedded in the file name, please submit the data file through the "My Proficiency Studies" tab located on the CTRP website following the instructions set forth above. The data will be stored anonymously, based on a randomly generated Assigned Data Set ID in a secured database for the study. All data will be treated in a confidential manner as set forth in the "Terms and Conditions for CTRP Proficiency Testing Programs," and agreed to by the participants.

Statistical Analysis

Estimates of the robust mean and robust standard deviation from applying Algorithm A within and between labs will be used for the computation of the repeatability standard deviation (s_r) and reproducibility standard deviation (s_R).

Through a stepwise statistical analysis of the data, a determination of Mandel's test statistics ***h*** and ***k*** for the individual participants will be conducted. Next, the Cochran's and the Grubb's tests test will be employed to identifier outliers. Using the estimates of the repeatability and reproducibility standard deviations, the standard deviation for proficiency testing, σ_{pt}, will be calculated in accordance with ISO 13528:2015. Participants' will be evaluated using the z-score, $z = \frac{x_i - x_{pt}}{\sigma_{pt}}$, where x_i is the robust mean of participant for a given measurand, x_{pt} is the assigned value for the proficiency test, and σ_{pt}, the standard deviation for the proficiency test. The Z-scores are commonly interpreted as

(i)	$\|Z\| \leq 2.0$	Satisfactory, acceptable
(ii)	$2.0 < \|Z\| < 3.0$	Questionable, a warning signal (W) is given
(iii)	$\|Z\| \geq 3.0$	Unsatisfactory, an action signal (A) is given.

A participant's ability to properly perform the analysis of the analytes of interest should only be based on the z-score for the parameters reported for the 1S4 and 3S3 certified smokeless reference

products. If an insufficient number of datasets are submitted for rigorous statistical evaluation, alternative scoring methods discussed in ISO 13528 will be employed and details will be provided in the interim report for participants to review and comment. The final report will contain the detailed approach for scoring performance.

Proficiency Test Coordinator

The Logistics Coordinator and Quality Manager for the Proficiency Testing Program for the Center for Tobacco Reference Products (CTRP) are listed in this section.

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For logistics (shipping, customs, etc.) concerns please contact the Logistics Coordinator:

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For analytical or reporting concerns please contact the Quality Manager:

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