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

CIG-2021A Proficiency Program Protocol

2021 Round 1

Objective

This round of testing will include analysis of the 1R6F certified reference cigarette and the 2R5F ultra-low yield reference cigarette. **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 cigarette filler properties to be analyzed are:

- Oven Volatiles
- pH
- Total Nicotine
- NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
- NNN (N-nitrosonornicotine)
- Arsenic (As)
- Cadmium (Cd)
- Ammonia (NH_3)

Due to the COVID-19 pandemic, dates are subject to change. Please check ctrp.uky.edu for updates during the study. To request an extension, please contact ruth.mcnees@uky.edu or ctrp@uky.edu.

Proficiency Study Timeframe

February 4, 2021: 09:00 AM EST	*PT round Opens, Test Kits available for purchase*
March 4, 2021: 09:00 AM EST	*Data submission portal Opens, First day of data submission*
April 29, 2021: 5:00 PM EDT	*Data submission portal Closes, Final day of data submission*
May 27, 2021	*Target date for issuance of Interim Report*
June 24, 2021	*Target date for issuance of Final Report, PT round Closes*

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

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

Test kits are available for purchase beginning on February 4, 2021. This round of testing for data submission will open on March 4, 2021 and close on April 29, 2021. 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 May 27, 2021. 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 be issued, tentatively,

on June 24, 2021. 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

Oven Volatiles (Moisture)	CORESTA N 76
pH	CORESTA N 69
Nicotine	CORESTA N 62
NNN (N-nitrosonornicotine)	CORESTA N 72, ISO/TS 22304:2008
NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone)	CORESTA N 72, ISO/TS 22304:2008
As (Arsenic)	
Cd (Cadmium)	
(NH_3) (Ammonia)	CORESTA N 79

Note: Not all analytes have a reference.

Proficiency Test Material (1R6F Certified Reference Cigarettes and 2R5F Ultra-low Yield Reference Cigarette)

Proficiency Test Material for this round of proficiency sampling 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 1R6F or 2R5F reference cigarettes from your inventory. Using the materials provided will ensure that all participants are using cigarettes from a batch that is pre-characterized or selected for the purposes of the Proficiency Test Scheme. Homogeneity of the 1R6F Proficiency Test Material was determined by selecting 12 random samples of the 1R6F certified reference cigarette and having them analyzed in at least triplicate. The testing was sub-contracted to a third-party laboratory meeting the quality requirements of the proficiency testing scheme in accordance with ISO/IEC 17043. Test results confirm that this Proficiency Test Material is fit for proficiency testing. Stability testing is on-going and 5 years of data show mean values to be stable within ±15% of the reference value from the Certificate of Analysis for the 1R6F certified reference cigarette available at the CTRP website (ctrp.uky.edu). The 2R5F Proficiency Test Material is a new reference cigarette, which has not been characterized for certified values.

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 cigarettes 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 historical data from previous rounds of testing, we anticipate participation from approximately 20 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 conditioning for the proficiency test.

Sample Equilibration and Handling

Prior to analysis, the cigarettes should be transferred from freezer to a refrigerator for a minimum of 24 hours or until the cigarettes are completely thawed, and then moved to room temperature for at least 2 hours until they reach temperature equilibrium.

For all tests (except the oven volatile test which uses non-ground cigarette filler) remove the tobacco filler from one carton of cigarettes, grind it to pass through 1 mm screen, place in a tight seal container and mix well.

Replicates Required

Replicates must be obtained **under repeatability conditions** i.e. same instrument and same operator**.**

Five independent replicates for each analyte is requested. Laboratories should follow their routine analysis method.

Expected values, and the metrological traceability and uncertainty, for selected analytes can be found in the Certificate of Analysis for the 1R6F certified reference cigarette. There is no Certificate of Analysis or posted values for the 2R5F Proficiency Test Materials. The results reported by participants will be scored using consensus values and the standard deviation of the proficiency study.

The analytes should be reported in units of on an as-is basis. Please report on as many analytes as you can. Note that only the mean values will be provided if there are less than 5 reporting labs for that analyte.

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. 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 section of the Excel reporting template for the cigarette type (1R6F or 2R5F). *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 the minimum significant digits requested 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 (CIG-2021A) 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 (mg/cig instead of g/cig), failure to calculate values for individual cigarettes, 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: *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.

Analysis Data

Please be sure to enter measurements for each analyte:

- *Measurements for each of the analytes (Oven Volatiles/Moisture, pH, Total Nicotine, NNN, NNK, As, Cd, and NH_3) for each of the 5 replicates.*
- *Please use the dropdown menu to select the "method" used in testing for Total Nicotine, NNN, NNK, As, Cd, and Ammonia. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	Total Nicotine	NNN	NNK	As	Cd	Ammonia
Method 1	MTBE method	LC/MS/ MS	LC/MS/ MS	ICP-MS	ICP-MS	IC
Method 2	Methanol method	GC/TEA	GC/TEA	AAS	AAS	
Enter alternate method (text)						

Names: MTBE – Methyl tert-Butyl Ether
LC/MS/MS: Liquid Chromatography-Tandem Mass Spectrometry
GC/TEA: Gas Chromatography – Thermal Energy Analyzer
ICP-MS – Inductively Coupled Plasma Mass Spectrometry
IC: Ion Chromatography
AAS: Atomic Absorption Spectroscopy

- *Please use the dropdown menu to select the "internal standard" used in testing for NNN and NNK. If your internal standard is not identified in the dropdown menu, please type your internal standard in the box provided.*

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

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

- *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 .

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 1R6F certified reference cigarette.

Proficiency Test Contacts

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

CTRP - Kentucky Tobacco & Research Development Center
1401 University Drive
Lexington, KY 40546-0236
CTRP@uky.edu

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

James T. Hall
Kentucky Tobacco & Research Development Center
1401 University Drive, Room B07
Lexington, KY 40546-0236
Phone: 859-257-2660
james.hall@uky.edu

For analytical or reporting concerns please contact the Quality Manager:

Ruth McNees
Kentucky Tobacco & Research Development Center
1401 University Drive, Room 200E
Lexington, KY 40546-0236
Phone: 859-257-9133
Ruth.mcnees@uky.edu