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

CIG-2025B Proficiency Program Protocol

2025 Round 2

Objective

This round of testing will include smoking the 1R6F reference cigarette using both the non-intense smoking regime (ISO 3308:2012) and the intense smoking regime (ISO 20778:2018) and the determination of physical properties. ***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 mainstream smoke measured properties are:

- NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone)
- NNN (N-nitrosonornicotine)
- NAT (N-nitrosoanatabine)
- NAB (N-nitrosoanabasine)
- Total Particulate Matter (TPM)
- Puff Count

Physical Properties that will be measured:

- Cigarette Resistance to Draw (pressure drop open)
- Cigarette Resistance to Draw (pressure drop closed)
- Filter Pressure Drop (fully encapsulated)
- Total Ventilation
- Filter Ventilation
- Tobacco Weight
- Cigarette Weight
- Air Permeability
- Firmness
- Circumference
- Cigarette Length
- Filter Plug Length
- Tipping Paper Length

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

March 27, 2025: 09:00 AM	*PT round Opens, Test Kits available for purchase*
May 1, 2025: 09:00 AM	*Data submission portal Opens, First day of data submission*
June 26, 2025: 5:00 PM	*Data submission portal Closes, Final day of data submission*
July 17, 2025	*Target date for issuance of Interim Report*
August 7, 2025	*Target date for issuance of Final Report, PT round Closes*

All times are Eastern Daylight Time (EDT) (New York, NY time)

Test kits are available for purchase beginning on March 27, 2025. This round of testing for data submission will open on May 1, 2025 and close on June 26, 2025. 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 July 17, 2025. 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 August 7, 2025. 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	ISO 3402:2023
TPM/ NFDPM	ISO 4387:2019, ISO 20779:2018
Intense Smoking Regime, Puff Parameters	ISO 20778:2018
Non-intense Smoking Regime, Puff	ISO 3308:2012, ISO 4387:2019
NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	CORESTA N 63 or 75, ISO/TS 22304:2008
NNN (N-nitrosonornicotine)	CORESTA N 63 or 75, ISO/TS 22304:2008
NAT (N-nitrosoanatabine)	CORESTA N 63 or 75, ISO/TS 22304:2008
NAB (N-nitrosoanabasine)	CORESTA N 63 or 75, ISO/TS 22304:2008
Resistance to Draw	ISO 6565:2015
Filter Pressure Drop	ISO 6565:2015
Total Ventilation	ISO 9512:2019
Filter Ventilation	ISO 9512:2019
Air Permeability	ISO 2965:2019
Circumference	ISO 2971:2013

Note: Not all smoking and physical parameters have a reference.

Proficiency Test Material (1R6F Reference Cigarettes)

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 reference cigarettes from your inventory.** Using the

1R6F reference materials provided will ensure that all participants are using cigarettes from a batch that is pre-characterized for the purposes of the Proficiency Test Scheme. Homogeneity of the Proficiency Test Material was determined by selecting 12 random samples 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 the 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).

General Guidance

Table 1 lists the smoking parameter and vent blocking specifications for each smoking regimen. The butt length for this testing is set at 35 mm or 1.38 inches.

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.

Table 1
Smoking parameter specifications

Smoking Regimen	*Puff Volume (mL)*	*Puff Interval (s)*	*Puff Duration (s)*	*Vent Blocking*
Non-intense	35 ± 0.3	60 ± 0.5	2 ± 0.02	0%
Intense	55 ± 0.5	30 ± 1.0	2 ± 0.02	100%

Note: Puff Interval is time in seconds from the start of one puff to the start of the next puff.[1]

Based on historical data from previous rounds of testing, we anticipate participation from approximately 25 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.

Participants should confirm the type of smoking machine being used (rotary or linear) and report the model and manufacturer. The temperature and relative humidity at the time the smoking is conducted should be recorded.

Test Item Storage

The samples should be stored in plastic bags at -20°C for long term storage and transferred to 4°C prior to conditioning for the proficiency test.

[1] 2012 CORESTA Collaborative Study for CORESTA Monitor #7 (CM7) for Determination of Test Piece, Weight, TPM, Water, Nicotine, NFDPM, Carbon Monoxide and Puff Count Obtained Under Mainstream ISO and Intense Smoking Regimes; May 2013

Sampling Equilibration and Handling

Samples should be conditioned for a minimum of 48 hours, but no more than 10 days at 22 ± 1°C and 60 ± 3% relative humidity before conducting each smoking parameter test.

Replicates Required

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

Smoking Parameter

Smoke 5 replicates of 1R6F for each smoking regime for both linear and rotary smoking machines. Laboratories should follow their routine smoking and 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 which is available at the CTRP website (ctrp.uky.edu). The results reported by participants for the 1R6F reference cigarettes will be scored using consensus values and the standard deviation of the proficiency study.

The analytes (NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, NNN (N-nitrosonornicotine), NAT (N-nitrosoanatabine), and NAB (N-nitrosoanabasine)) should be reported in units of ng/cig 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.

Physical Testing

For each physical parameter, 5 replicates of the mean of the measurements of 20 cigarettes should be recorded. Results will be presented as consensus values based on participant results.

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 purchase of a Proficiency Test Kit. If both a linear and a rotary Proficiency Test Kit or multiple kits for each machine are ordered, the Excel reporting template for each of the Proficiency Test Kits must be downloaded. Each Excel reporting template has a unique "Assigned Data Set ID" based on a customer's purchase. Please make sure that data into the correct Excel reporting template (linear or rotary) for each kit purchased. *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.* 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. Participants are encouraged to provide the data collected for each round of testing without discussing the results with other potential participants.

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-2025B) 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: *linear_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.

Machine Smoking Data

Please be sure to enter data for the specific smoking machine (linear or rotary) used in your analysis:

- *Smoking Machine Make (i.e., manufacturer)*
- *Smoking Machine Model*
- *Enter any notes on data collection (if necessary)*

Non-Intense Data

Please be sure to enter measurements for the specific Non-Intense Data:

- *Non-Intense Data Test Date*
- *Linear Machines: Enter the number of ports used per replicate.*
  Rotary Machines: Enter the number of collections per replicate.
- *Linear Machines: Enter the number of cigarettes smoked per port.*
  Rotary Machines: Enter the number of cigarettes per collection.
- *Laboratory conditions (6 variables) for each of replicates*
- *Measurements for each of the 4 smoking parameters (NNK, NNN, NAT, and NAB) for each of the 5 replicates.*
- *Please use the dropdown menu to select the "method" used in testing for 4 smoking parameters. 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)				

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

- *Please type the "internal standard" used in testing for each of the 4 smoking parameters.*

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

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

- *Measurements for Total Particulate Matter and Puff Count.*
- *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.*
- *For measurements that are below the Limit of Detection (LOD) or Limit of Quantification (LOQ), enter -1 in the Excel cell.*

Intense Smoking Data

Please be sure to enter measurements for the specific Intense Smoking Data:

- *Intense Test Date*
- *Linear Machines: Enter the number of ports used per replicate.*
  Rotary Machines: Enter the number of collections per replicate.
- *Linear Machines: Enter the number of cigarettes smoked per port.*
  Rotary Machines: Enter the number of cigarettes per collection.
- *When smoking is done using the Intense smoking regime, please be sure to enter data for the Ventilation Blocking Method.*
- *Laboratory conditions (6 variables) for each of replicates.*
- *Measurements for each of the 4 smoking parameters (NNK, NNN, NAT, and NAB) for each of the 5 replicates.*
- *Measurements for Total Particulate Matter and Puff Count.*
- *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.*
- *For measurements that are below the Limit of Detection (LOD) or Limit of Quantification (LOQ), enter -1 in the Excel cell.*

Physical Measurement Data

There are 5 data-entry rows (replicates 1 to 5) for each of the physical parameters. Determine the individual measurements (20 cigarettes for each replicate) and report the average. There are 13 physical parameters and associated standard deviations for each. These rows and cells should not be altered, as they are required for data import and transformation.

Please be sure to enter the following data for each of the 13 physical measurements:

- Physical Measurements Machine Make (i.e., manufacturer)
- Physical Measurements Machine Model
- Physical Measurements Test Date
- No adjustments should be made to the physical data collected
- 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}, and the uncertainty of the consensus mean will be calculated in accordance with ISO 13528:2022. 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 consensus mean 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. 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 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
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Lexington, KY 40546-0236
Phone: 859-257-2660
james.hall@uky.edu

For analytical or reporting concerns please contact the Quality Manager:

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