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

CIG-2022A Proficiency Program Protocol

2022 Round 1

Objective

This round of testing will include smoking the 1R6F reference cigarette using both the International Organization for Standardization (ISO) and the Health Canadian Intense (HCI) regimes. **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:

- o-toluidine
- 2,6-dimethylanilin
- o-anisidine
- 1-aminonaphthalene
- 2-aminonaphthalene
- 3-aminobiphenyl
- 4-aminobiphenyl
- Total Particulate Matter
- Puff Count
- Benzo [α] pyrene (BaP)
- BaP-Total Particulate Matter (BaP-TPM)
- BaP-Puff Count

Proficiency Study Timeframe

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.

January 13, 2022: 09:00 AM EST	*PT round Opens, Test Kits available for purchase*
February 24, 2022: 09:00 AM EST	*Data submission portal Opens, First day of data submission*
April 14, 2022: 5:00 PM EDT	*Data submission portal Closes, Final day of data submission*
May 5, 2022	*Target date for issuance of Interim Report*
June 2, 2022	*Target date for issuance of Final Report, PT round Closes*

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

Test kits are available for purchase beginning on January 12, 2022. This round of testing for data submission will open on February 24, 2022 and close on April 14, 2022. 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 5, 2022. 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 2, 2022. 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 3402:1999
Total Particulate Matter	ISO 4387:2000
Aromatic Amines Analysis	CORESTA N 95
Intense Smoking Regime, Puff Parameters	Health Canada Method T-115, ISO 20778:2018
ISO Smoking Regime, Puff Parameters	ISO 3308:2012, ISO 4387:2000
Benzo [α] pyrene	ISO 22634:2019, CORESTA N 58

Note: Not all smoking 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 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.

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.

Based on historical data from previous rounds of testing, we anticipate participation from approximately 15-20 tobacco research/analytical 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.

Table 1
Smoking parameter specifications

Smoking Regimen	*Puff Volume (mL)*	*Puff Interval (s)*	*Puff Duration (s)*	*Vent Blocking*
ISO	35 ± 0.3	60 ± 0.5	2 ± 0.02	0%
HCI	55 ± 0.5	30 ± 1	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]

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 4°C prior to conditioning for the proficiency test.

Conditioning

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 and physical parameter test.

Replicates Required

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

Smoking Parameter
Smoke 5 replicates for each smoking regime for both linear and rotary smoking machines. Laboratories should follow their routine smoking plan.

The analytes should be reported in units of ng/cig, on an as-is basis, except TPM, which is reported in mg/cig. 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. If you order a linear and a rotary Proficiency Test Kit or multiple kits for each machine, 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 (linear or rotary). *Please note that there is no need to 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

[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 HCI Smoking Regimes; May 2013

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-2022A) 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 University of Kentucky 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)*

ISO Data – Aromatic Amines

Please be sure to enter measurements for the specific ISO Data:

- *ISO 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 7 smoking parameters (o-toluidine, 2,6-dimethylanilin, o-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl and 4-aminobiphenyl) for each of the 5 replicates.*
- *Please use the dropdown menu to select the "method" used in testing for 7 smoking parameters. If your method is not identified in the dropdown menu, please type your method in the box provided.*
- *Please type your derivative reagent used in the box provided.*

	o-toluidine	2,6-dimethylanilin	o-anisidine	1-aminonapthalene	2-aminonapthalene	3-aminobiphenyl	4-aminobiphenyl
Method 1	GC/MS	GC/MS	GC/MS	GC/MS	GC/MS	GC/MS	GC/MS
Method 2	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS
Enter alternate method (text)							
Derivative reagent							

Names: LC/MS/MS: Liquid Chromatography-Tandem Mass Spectrometry
GC/MS: Gas Chromatography – Mass Spectrometry

- *Please type the "internal standard" used in testing for each of the 7 smoking parameters.*

	o-toluidine	2,6-dimethylanilin	o-anisidine	1-aminonapthalene	2-aminonapthalene	3-aminobiphenyl	4-aminobiphenyl
Internal Standard (enter text)							

- 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.

ISO Data – Benzo[α]pyrene (BaP)

Please be sure to enter measurements for the specific ISO Data:

- *ISO Data Test Date*
- *Linear Machines: Enter the number of ports used per replicate.*

Rotary Machines: Enter the number of collections used 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 BaP of the 5 replicates.*
- *Please use the dropdown menu to select the "instrument" used in testing for BaP measurement.*
- *Please use the dropdown menu to select the "method" used in testing for BaP smoking parameter. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	BaP
Method 1	GC/MS
Method 2	LC/FLD
Enter alternate method (text)	

LC/FLD: Liquid Chromatography-Fluorescence Detector
GC/MS: Gas chromatography–mass spectrometry

- *Please use the dropdown menu to select the "internal standard" used in testing for BaP smoking parameters. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	BaP
Internal Standard Option 1	BaP-d12
Other (enter text)	

- *Measurements for BaP Total Particulate Matter and BaP 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.*

<u>Intense Smoking Data – Aromatic Amines</u>

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 HCI 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 7 smoking parameters (o-toluidine, 2,6-dimethylanilin, o-anisidine, 1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl and 4-aminobiphenyl) 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.

Intense Smoking Data – Benzo[α]pyrene (BaP)

- *Intense Test Date*
- *Linear Machines: Enter the number of ports used per replicate.*
  Rotary Machines: Enter the number of collections used 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 HCI smoking regime, please be sure to enter data for the Ventilation Blocking Method*
- *Laboratory conditions (6 variables) for each of replicates*
- *Measurements for BaP of the 5 replicates.*
- *Measurements for BaP Total Particulate Matter and BaP 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.*

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. 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:

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Lexington, KY 40546-0236
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james.hall@uky.edu

For analytical or reporting concerns please contact the Quality Manager:

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1401 University Drive, Room 200E
Lexington, KY 40546-0236
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