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

CIG-2022C Proficiency Program Protocol

2022 Round 3

Objective

This round of testing will include smoking the 1R6F reference cigarette using both the non-intesnse smoking regime (ISO 3308:2012) and the intense smoking regime (ISO 20778:2018). ***Please note that there is no need to 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:

- Formaldehyde
- Acetaldehyde
- Acetone
- Acrolein
- Propionaldehyde
- Crotonaldehyde
- 2-butanone
- n-butyraldehyde
- 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

June 2, 2022: 09:00 AM EDT	*PT round Opens, Test Kits available for purchase*
July 7, 2022: 09:00 AM EDT	*Data submission portal Opens, First day of data submission*
September 1, 2022: 5:00 PM EDT	*Data submission portal Closes, Final day of data submission*
September 22, 2022	*Target date for issuance of Interim Report*
October 13, 2022	*Target date for issuance of Final Report*

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

Test kits are available for purchase beginning on June 2, 2022. This round of testing for data submission will open on July 7, 2022 and close on September 1, 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 September 22, 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 October 13, 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
Formaldehyde	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
Acetaldehyde	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
Acetone	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
Acrolein	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
Propionaldehyde	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
Crotonaldehyde	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
2-butanone	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
n-butyraldehyde	ISO 21160:2018 / ISO 23922:2020/ CORESTA N 74
Intense smoking regime, puff parameters	ISO 20778:2018
Non-intense smoking regime, puff parameters	ISO 3308:2012
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 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 reports. 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 previous rounds of testing, participation is expected to include about 20 participants from all interested laboratories. Participants that do not receive a proficiency testing kit or receive a damaged kit are encouraged to contact the CTRP (ctrp@uky.edu) immediately to ensure the participants ability to continue to participate in the Proficiency Test Scheme.

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.

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	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]

[1] 2021 Collaborative Study of CORESTA Monitor 9 (CM9) for the Determination of Test Piece Weight, TPM, Water, Nicotine, NFDPM, Carbon Monoxide and Puff Count Obtained under Mainstream 'Non-Intense' and 'Intense' Smoking Regimes; March 2022

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.

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 and analytical methods. 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 at the CTRP website (ctrp.uky.edu). Results will be presented as consensus values and the standard deviation based on participant results.

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 and the standard deviation based on participant results.

The analytes should be reported in units of µg/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.

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 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-2022C) 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)*

Non-intense Data

Please be sure to enter measurements for the specific Non-intense Smoking 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 8 smoking parameters (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone and n-butyraldehyde) for each of the 5 replicates.*
-
- *Please use the dropdown menu to select the "method" used in testing for each of the 8 smoking parameters. If your method is not identified in the dropdown menu, please type your method in the box provided.*

	formaldehyde	acetaldehyde	acetone	acrolein	propionaldehyde	crotonaldehyde	2-butanone	n-butyraldehyde
Method 1	HPLC-UV	HPLC-UV	HPLC-UV	HPLC-UV	HPLC-UV	HPLC-UV	HPLC-UV	HPLC-UV
Method 2	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS	GC-MS
Enter alternate method								

For the instrument: GC- Gas Chromatography, HPLC- High Performance Liquid Chromatography

- *Please type your internal standard in the box provided.*

	formaldehyde	acetaldehyde	acetone	acrolein	propionaldehyde	crotonaldehyde	2-butanone	n-butyraldehyde
Internal Standard								
Derivatizing Reagent								

- Measurements for 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

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 8 smoking parameters (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone and n-butyraldehyde) for each of the 5 replicates.*
- Measurements for 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.

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 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).

It is noted herein that only the mean values are provided if there are less than 5 reporting labs for any analyte.

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.

Proficiency Test Coordinator

The CTRP staff 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 CTRP staff:
James Hall
Kentucky Tobacco & Research Development Center
1401 University Drive, Room B07
Lexington, KY 40546-0236
Phone: (859) 257-9318
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