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Comparison of Select Analytes from Different Laboratories in Tobacco and Smoke for Commercial Cigars Across a Range of Designs *

by

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SUMMARY

Analytical research relevant to cigar products is relatively limited compared to other tobacco product categories and very few standardized methods are in place. In recent years, scientific and regulatory interests in cigar testing have increased. Thus, there is a need for increased foundational knowledge in the product space. The objective of this work was to characterize cigars across a range of design features to understand relative magnitude of results and to evaluate analytical variability among and between three laboratories using their own practices and methodology.

Commercial cigars across a broad range of design characteristics were evaluated for tobacco and smoke harmful or potentially harmful constituents (HPHCs) typically applied to cigarettes and smokeless tobacco products (i.e., FDA-Center for Tobacco Product's abbreviated list). Design features such as size, manufacturing technique (machine *vs.* hand), and tipping (untipped, filtered, and mouthpiece designs) were included in the study matrix. For smoke analytes, a currently accepted regime for cigar smoking was employed. Cigars from the same lots were tested by three independent laboratories using their own methodologies to assess lab-to-lab differences across 5 physical parameter measures, 10 tobacco analytes, and 21 smoke analytes. Thus, the overall study was a randomized design with a two-factor arrangement: cigar type (with six levels) and laboratory (with three levels). Calculation of Lab Product Range (LPR) allowed for a comparison of magnitude in results across the product set. Data analysis included determination of relative variability (% RSD, Relative Standard Deviation) for replicate testing among the products. Lab Range (LR) was calculated to compare spread in results among the laboratories for each product. Low LR and similar LPR among the labs is an indicator of laboratory consistency.

LPR results ranged from 45% for length to 445% for smoke ammonia. For example, tobacco weight, at approximately 260% LPR, ranged from 1 g/cigar to 18 g/cigar. Tobacco NNN, LPR 328%, ranged from 2000 ng/g to 27,000 ng/g. Replicate variability (% RSD) of physical characteristics, tobacco, and smoke analytes was much higher for the cigar products than previously reported for typical cigarette products. For example, tobacco weight % RSD was as high as 11%. Commercial factory-made cigarettes are typically reported at < 1% RSD.

Lab Range was as low as < 1% for cigar length and as high as 247% for tobacco NNK. Smoke nicotine, one of the few analytes tested using standard methodology and subject to routine collaborative studies, had an LR as high as 73%. Tobacco BaP and carbonyls LR results were very inconsis-

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tent between labs. For example, acetaldehyde for Sample D was reported as 0.29, 0.81, and < 0.10 µg/g for Lab 1, Lab 2, and Lab 3, respectively.
Cigars with a broad range of design features were shown to have relatively high variability, a wide magnitude in analyte levels and smoking characteristics, and in some cases quite distinct results from lab-to-lab. Some analytes, such as BaP and crotonaldehyde, were determined to be inadequate for characterization in cigar tobacco due to a lack of quantifiable results. Based on LR results, smoke ammonia and carbonyls were found to lack robust methodology.
The results of this study support the need for increased standardization for smoking analyte methods and the use of reference products across studies to improve understanding of product differences and the contribution of analytical variability. Additionally, in the absence of foundational data, the results are cautionary regarding using isolated data sets for characterization *via* HPHC testing for cigars, especially hand rolled products. [Contrib. Tob. Nicotine Res. 33 (2024) 234–252]

KEYWORDS

Cigar, HPHC (harmful or potentially harmful constituents), FDA Center for Tobacco Products, MMC (machine-made cigar), TNCO ("tar", nicotine, carbon monoxide), tobacco, premium cigar.

ZUSAMMENFASSUNG

Die analytische Forschung im Bereich von Zigarren ist im Vergleich zu anderen Kategorien von Tabakerzeugnissen relativ begrenzt, und es gibt nur sehr wenige standardisierte Methoden. In den letzten Jahren hat das wissenschaftliche und regulatorische Interesse an Zigarrentests zugenommen. Es besteht daher ein Bedarf an mehr Grundlagenwissen in diesem Produktbereich. Ziel dieser Arbeit war die Charakterisierung von Zigarren über eine Reihe von Designmerkmalen hinweg, um die relative Größenordnung der Ergebnisse zu verstehen und die analytische Variabilität zwischen drei Laboratorien, die ihre eigenen Verfahren und Methoden anwenden, zu bewerten.
Kommerzielle Zigarren mit einem breiten Spektrum an Designmerkmalen wurden auf die für Zigaretten und rauchlose Tabakerzeugnisse typischerweise verwendeten besonders besorgniserregenden Eigenschaften von Tabak und Tabakrauch (d. h. die verkürzte Liste der FDA-HPHC) untersucht. Designmerkmale wie Größe, Herstellungstechnik (maschinell oder manuell) und Mundstück (ohne oder mit Filter, mit Mundstückdesigns) wurden in die Studienmatrix aufgenommen. Für die Rauchanalytik wurde ein derzeit anerkanntes Verfahren für das Zigarrenrauchen angewandt. Zigarren aus denselben Chargen wurden von drei unabhängigen Labors mit ihren eigenen Methoden getestet, um die Unterschiede zwischen den Labors bei 5 physikalischen Parametern, 10 Tabakanalyten und 21 Rauchanalyten zu bewerten. Die gesamte Studie war also ein randomisiertes Design mit einer Zwei-Faktoren-Anordnung: Zigarrentyp (mit sechs Stufen) und Labor (mit drei Stufen). Die Berechnung der Laborproduktspanne (LPR) ermöglichte einen Vergleich der Größenordnung der Ergebnisse innerhalb der Produktgruppe. Die Datenanalyse umfasste die Bestimmung der relativen Variabilität (% RSD) für Wiederholungstests zwischen den Produkten. Der Laborbereich (LR) wurde berechnet, um die Streuung der Ergebnisse zwischen den Labors für jedes Produkt zu vergleichen. Eine niedrige LR und eine ähnliche LPR zwischen den Labors ist ein Indikator für die Konsistenz der Labors.
Die LPR-Ergebnisse reichten von 45% für die Zigarrenlänge bis zu 445% für Rauchammoniak. So reichte beispielsweise das Tabakgewicht bei einer LPR von etwa 260% von 1 g/Zigarre bis 18 g/Zigarre. Tabak NNN, LPR 328%, reichte von 2000 ng/g bis 27.000 ng/g. Die Wiederholungsvariabilität (% RSD) der physikalischen Merkmale, des Tabaks und der Rauchanalyten war bei den Zigarrenprodukten viel höher als bei den typischen Zigarettenprodukten. Die % RSD des Tabakgewichts lag beispielsweise bei bis zu 11 %. Für handelsübliche, fabrikmäßig hergestellte Zigaretten wird in der Regel eine RSD von < 1% angegeben.
Die Laborwerte lagen zwischen < 1% für die Zigarrenlänge und 247% für den NNK-Wert des Tabaks. Bei Rauchnikotin, einem der wenigen Analyten, die nach einer Standardmethodik getestet wurden und Gegenstand von routinemäßigen Kooperationsstudien sind, lag die LR bei 73%. Die LR-Ergebnisse für BaP und Carbonyle im Tabak waren von Labor zu Labor sehr uneinheitlich. Zum Beispiel wurde Acetaldehyd für Probe D mit 0,29, 0,81 und < 0,10 µg/g für Labor 1, Labor 2 und Labor 3 angegeben.
Es zeigte sich, dass Zigarren mit einem breiten Spektrum an Designmerkmalen eine relativ hohe Variabilität, eine große Bandbreite an Analyten und Rauchmerkmalen und in einigen Fällen recht unterschiedliche Ergebnisse von Labor zu Labor aufweisen. Einige Analyten, wie BaP und Crotonaldehyd, wurden als ungeeignet für die Charakterisierung von Zigarrentabak eingestuft, da keine quantifizierbaren Ergebnisse vorlagen. Auf der Grundlage von LR-Ergebnissen wurde festgestellt, dass es bei Rauchammoniak und Carbonylverbindungen keine zuverlässige Methodik gibt.
Die Ergebnisse dieser Studie unterstreichen die Notwendigkeit einer stärkeren Standardisierung der Methoden für Rauchanalytik und der Verwendung von Referenzprodukten in verschiedenen Studien, um das Verständnis für Produktunterschiede und den Beitrag der analytischen Variabilität zu verbessern. Darüber hinaus sind die Ergebnisse in Ermangelung grundlegender Daten mit Vorsicht zu betrachten, was die Verwendung isolierter Datensätze für die Charakterisierung von Zigarren, insbesondere von handgerollten Produkten, durch HPHC-Tests angeht. [Contrib. Tob. Nicotine Res. 33 (2024) 234–252]

RESUME

La recherche analytique dans le domaine des cigares est relativement limitée par rapport à d'autres catégories de produits du tabac, et il existe très peu de méthodes normalisées. Ces dernières années, l'intérêt scientifique et réglementaire pour les tests de cigares s'est accru. Il est donc

nécessaire d'acquérir davantage de connaissances fondamentales dans ce domaine de produits. L'objectif de ce travail était de caractériser les cigares sur une série de caractéristiques de construction afin de comprendre l'ordre de grandeur relatif des résultats et d'évaluer la variabilité analytique entre trois laboratoires utilisant leurs propres procédures et méthodes.
Des cigares commerciaux présentant un large éventail de caractéristiques de conception ont été étudiés pour les propriétés extrêmement préoccupantes du tabac et de la fumée de tabac (c'est-à-dire la liste abrégée des HPHC de la FDA) typiquement utilisées pour les cigarettes et les produits du tabac sans fumée. Les caractéristiques de conception telles que la taille, la technique de fabrication (mécanique ou manuelle) et l'équipement (non équipé, filtré et conception de l'embout) ont été incluses dans la matrice d'étude. Pour l'analyse de la fumée, une méthode actuellement reconnue pour fumer des cigares a été utilisée. Des cigares provenant des mêmes lots ont été testés par trois laboratoires indépendants avec leurs propres méthodes afin d'évaluer les différences entre les laboratoires pour 5 paramètres physiques, 10 analyses de tabac et 21 analyses de fumée. L'ensemble de l'étude était donc une conception randomisée avec une disposition à deux facteurs : type de cigare (avec six niveaux) et laboratoire (avec trois niveaux). Le calcul de la marge de produit de laboratoire (LPR) a permis de comparer l'ordre de grandeur des résultats au sein du groupe de produits. L'analyse des données a consisté à déterminer la variabilité relative (% RSD) pour les tests répétés entre les produits. L'étendue du laboratoire (LR) a été calculée afin de comparer la dispersion des résultats entre les laboratoires pour chaque produit. Une LR faible et une LPR similaire entre les laboratoires sont des indicateurs de la cohérence des laboratoires.
Les résultats LPR allaient de 45% pour la longueur à 445% pour l'ammoniac de fumée. Par exemple, pour un LPR d'environ 260%, le poids du tabac variait de 1 g/cigare à 18 g/cigare. Le tabac NNN, LPR 328%, allait de 2000 ng/g à 27 000 ng/g. La variabilité de la répétition (% RSD) des caractéristiques physiques, du tabac et des analytes de fumée était beaucoup plus élevée pour les produits à base de cigare que pour les produits typiques à base de cigarette. Le % RSD du poids du tabac, par exemple, pouvait atteindre 11%. Pour les cigarettes commerciales fabriquées en usine, on indique généralement un RSD de < 1%.
Les valeurs de laboratoire allaient de < 1% pour la longueur du cigare à 247% pour la valeur NNK du tabac. Pour la nicotine fumée, l'un des rares analytes testés selon une méthodologie standard et faisant l'objet d'études de coopération de routine, la LR était de 73%. Les résultats de LR pour le BaP et les carbonyles dans le tabac étaient très variables d'un laboratoire à l'autre. Par exemple, l'acétaldéhyde pour l'échantillon D a été rapporté à 0,29, 0,81 et < 0,10 µg/g pour le laboratoire 1, le laboratoire 2 et le laboratoire 3.
Il est apparu que les cigares présentant un large éventail de caractéristiques de conception présentaient une variabilité relativement élevée, un large éventail d'analytes et de caractéristiques de fumée et, dans certains cas, des résultats assez différents d'un laboratoire à l'autre. Certains analytes, tels que le BaP et le crotonaldéhyde, ont été jugés inappropriés pour la caractérisation du tabac à cigares en raison de l'absence de résultats quantifiables. Sur la base des résultats de LR, il a été constaté qu'il n'existait pas de méthodologie fiable pour l'ammoniac de fumée et les composés carbonylés.
Les résultats de cette étude soulignent la nécessité d'une plus grande standardisation des méthodes d'analyse des fumeurs et de l'utilisation de produits de référence dans différentes études afin d'améliorer la compréhension des différences entre les produits et la contribution de la variabilité analytique. En outre, en l'absence de données de base, les résultats doivent être considérés avec prudence en ce qui concerne l'utilisation d'ensembles de données isolés pour la caractérisation des cigares, en particulier des produits roulés à la main, par des tests HPHC. [Contrib. Tob. Nicotine Res. 33 (2024) 234–252]

INTRODUCTION

Cigars as a consumer product category represent a wide range of design and smoking characteristics. Products may be smaller than a conventional cigarette (i.e., < 8 mm) or greater than 25 mm in diameter for a smokable cigar. They may be machine- or hand-made. The simplest cigar design is a cylinder of tobacco with a tobacco leaf wrapper. However, cigars may have filters, mouthpieces, or reconstituted leaf wrappers and may be non-cylindrical in shape. When smoked under machine conditions, smoking times may vary from several minutes to several hours, with puff counts as high as several hundred.
While there are decades of characterization studies and numerous standardized analytical procedures for conventional cigarettes, relatively little published analytical data exists for cigar products (1). Previous product comparison studies consistently highlight this product category's inherent natural variability and the numerous testing challenges, especially concerning the evaluation of smoke constituents. Furthermore, few standardized test methods (2) exist, particularly for smoke analysis, and reference products have only been available for the product category since 2017 (3). In 2016, FDA extended regulatory authority to include cigars but with no explicit list of harmful and potentially harmful constituents (HPHCs) required for regulatory reporting (4, 5). There are limited studies for laboratory comparison for smoke methods, including an annual "tar", nicotine, and carbon monoxide (TNCO) collaborative study and one recent study to establish repeatability and reproducibility (*r* and *R*) values for smoke tobacco-specific nitrosamines (TSNA) and benzo-a-pyrene (BaP) (6, 7). One may also see from these studies that few laboratories have expertise in cigar smoking. TNCO studies are typically made of 5–12 participating labs while the smoke BaP study had 4 participants.
Thus, the purpose of this study was to evaluate cigar products across a wide range of typical design features for selected constituents to increase our understanding of the product category and the testing landscape. Specifically, the study focus was on the relative magnitude of analytical results, analytical product variability, and lab-to-lab differences. The testing was conducted at three ISO 17025-accredited laboratories using methods, procedures and practices, and

Table 1. Sample information and nominal physical parameters values.

Study ID	Product Type	Circumference (mm)	Diameter (mm)	Length (mm)	Weight (g)	Features of note
A	Large filter cigar	22	7	99	with filter: 1.29	Filtered
B	Small cigar	25	8	99	2.57	Irregular lighting end
C	Cigarillo	25	8	114	2.87	Tapered mouth-end
D	Wood-tipped cigar	28	9	with tip: 120 without tip: 100	with tip: 3.90 without tip: 2.33	Non-cylindrical wooden mouth-piece
E	Hand-made cigar	65	20.7	127	14.5	Closed mouth-end, requires cutting
F	Hand-made cigar	73	23	152	18.5	Closed mouth-end, requires cutting

reference or control samples in routine use in their facilities. The results of this study will demonstrate how the lack of standardized methodology impacts testing and reporting of cigar physical parameters and HPHC results, especially across different laboratories. Without standardization, comparison of cigar HPHC results between studies would potentially lead to erroneous conclusions. Products selected for the study were exemplary of typical design features that may present different analytical challenges, including non-cylindrical shape, tapered mouth-ends, an uneven lighting end, relatively large diameter, and tobacco with flavors.
Lot-matched samples were sent to each laboratory included in the study. A key objective was to assess differences in *in-practice* methodology between laboratories, so prescriptive instructions were limited to reporting requirements.
The products used in this study were evaluated for content and/or delivery for selected constituents typical of requirements for cigarette and/or smokeless tobacco testing, including nicotine, carbon monoxide (CO), carbonyls, volatile organic compounds (volatiles), metals, TSNAs, polyaromatic amines (PAA), and polyaromatic hydrocarbons (PAH). Many of these constituents are listed in industry guidance issued by the FDA that includes reporting obligations for HPHCs in cigarette filler and smoke and/or smokeless tobacco products under section 904(a)(3) of the 2009 Family Smoking Prevention and Tobacco Control Act (8). Physical parameter measurements such as length and weight were included in the study as indicators for the inherent production variability of each product.
For TNCO testing, the cigars were smoked under a set of industry-developed smoking methods (9–11). These methodologies were designed to maintain a constant airflow through the cigar during smoking to account for the relatively wide range of diameters, tobacco weight, burn times, and puff counts expected across this broad product category. The equipment specifications and puffing parameters for these methodologies were applied to the collection of cigar smoke vapor and/or condensate to test for the other smoke analytes in the study.

Figure 1. Cigar products tested. Samples are ordered from top to bottom in the photograph as Sample A, Sample B, Sample C, Sample D, Sample E, and Sample F.

MATERIALS AND METHODS

Study design

The study design was a randomized two-factor arrangement. The two major factors were: Products (with six levels defined by size or weight, making technique and tipping) and Laboratory (with three levels as Lab 1, 2, 3). Product selection was based on inclusion of typical product types with standard and challenging design parameters (e.g., cylindrical *vs.* non-cylindrical designs). Laboratory selection criteria included capability and expertise to test for all product types and analytes and measures, and use of on-scope for ISO 17025 methodology to conduct the study. Note, our original target was five laboratories. No other labs evaluated for the study met all criteria for inclusion.

Materials

Six conventional cigar products were purchased through wholesale or retail sources for testing. Information for each of the products is listed in Table 1 and the products are displayed in Figure 1. The physical measures noted were estimated based on testing a small aliquot of each product during the development of the study design.

Methods

ISO 17025-accredited laboratories used *in-house* analytical methodology to evaluate the cigar samples for selected HPHCs. Typically, methods used were adaptations of, or

similar in principle to, existing cigarette or smokeless tobacco methods referenced herein.

Non-reportable values

No requirements for target method limits of quantitation (LOQ) or reporting conventions for data outside a method's upper or lower limits were provided to participating labs. For samples with one or more < LOQ values, post-reporting calculations were based on the reported average.

Physical parameters

Cigar weight, tobacco weight, cigar length, diameter, and resistance to draw (n = 20) were measured using conventional equipment in everyday use in industry laboratories with modifications as warranted by laboratories based on unique features of a given sample (12, 13).

Tobacco testing methods

Tobacco was isolated from other cigar components in preparation for analysis. As warranted, tobacco samples were oven-dried for consistent grinding; thus, analytes may be reported on an 'as-is' or a dry weight basis. Replicate analysis (n = 7) was conducted on separate-grind aliquots.
Nicotine: Ground tobacco was extracted using liquid/liquid extraction into organic solvent followed by gas chromatography with a downstream flame ionization detector (GC-FID) analysis (14) (Lab 2, Lab 3) or using aqueous solvent followed by continuous flow analysis (15) (Lab 1).
Ammonia: Ground tobacco was extracted using dilute acid. Filtered extracts were analyzed by ion chromatography (IC) (16).
Carbonyls: Acetaldehyde, crotonaldehyde, formaldehyde; tobacco aliquots were extracted and derivatized in a two-phase system with aqueous buffer and isohexane using 2,4-dinitrophenyl hydrazine (DNPH) as a derivatization agent. Extracts were subsequently analyzed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (17).
Metals: Arsenic and cadmium; digested tobacco was analyzed by inductively coupled plasma mass spectroscopy (18).
Polyaromatic hydrocarbons (PAH): Benzo[a]pyrene (BaP); tobacco was extracted in methanol followed by solid phase extraction (SPE) clean-up and analysis by gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode (19).
Tobacco-specific nitrosamines (TSNAs): N-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); ground tobacco was extracted using dilute ammonium acetate, and extracts were analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS) (20).

Smoke testing methods

Smoke collection and trapping: Cigar preparation and machine smoking requirements are described in CORESTA Recommended Method CRM 65 (11). As prescribed, one or two cigars were smoked per replicate (n = 7). Smoke was collected onto 44-mm, 55-mm, or 92-mm glass fiber filter pads with > 99% particulate trapping efficiency for each replicate analysis. For carbonyls, smoke was collected directly by two in-series impingers. For volatile organic compounds (VOC) determinations, single chilled impingers were placed in-line with the filter pads.
Method quality control measures were as-typical for each laboratory and included testing an *in-house* control chart sample or analyte-spiked matrix samples.
"Tar", nicotine, carbon monoxide (TNCO): Total particulate matter (TPM) was determined gravimetrically. Filter pads were subsequently extracted with alcohol, extracts were analyzed by GC-FID for determination of nicotine and gas chromatography with a thermal conductivity detector (GC-TCD) for determination of water (21, 22). Nicotine-free dry particulate matter, "tar", was calculated by subtracting nicotine and water values from the TPM value. Carbon monoxide was determined concurrently with smoke collection for nicotine and water using a tandem non-dispersive infrared (NDIR) analyzer (23).
Ammonia: Cigars were smoked through glass filter pads with in-line impingers containing dilute acid. Pads were extracted with the impinger solvent. Extracts were analyzed by ion chromatography (IC) (24).
Carbonyls: Acetaldehyde, acrolein, crotonaldehyde, formaldehyde; mainstream cigar smoke was trapped in impingers containing an acidified 2,4-dinitrophenylhydrazine (DNPH) solution. After smoking, trizma base was added to the combined solutions, which were subsequently analyzed by ultra performance liquid chromatography with a diode array detector (UPLC-DAD) (25).
Polyaromatic amines (PAA): 4-Aminobiphenyl, 1-aminonaphthalene, 2-aminonaphthalene; pads were extracted with dichloromethane followed solid phase extraction (SPE) clean-up and analysis by GC-MS in negative chemical ionization (NCI) mode (26).
Polyaromatic hydrocarbons (PAH): Benzo[a]pyrene (BaP); BaP analysis was conducted by extraction in methanol followed by SPE clean-up and analysis by GC-MS in -selected ion monitoring (SIM) mode (27).
Tobacco-specific nitrosamines (TSNAs): NNN, NNK; cigars were smoked onto glass filter pads which were subsequently extracted using dilute ammonium acetate. Extracts were analyzed by LC-MS/MS (28).
Volatile organic compounds (VOC): Acrylonitrile, benzene, 1,3-butadiene, isoprene, toluene; filter pads were extracted in methanol (MeOH), and extracts were analyzed by GC-MS in SIM mode (29).

Statistical analysis

The results obtained from these analyses were tabulated as mean ± one standard deviation for levels of selected compounds. As indicated, Analysis of Variance (ANOVA) was used to compare data among laboratories. Statistical analyses were performed using JMP 13.0.0 (SAS Institute, Inc. Cary, NC, USA). The significance level was established as $p < 0.05$ for all comparisons.
Data from each laboratory were compiled and evaluated to determine the range in mean among the sample set, the relative variability (% RSD, Relative Standard Deviation) among the sample types, and to generally compare the

laboratories' results typically through evaluation of relative range values. As a practical method for laboratory comparisons, two types of relative range were calculated and expressed as a percentage value and without regard for standard deviation or statistical significance as noted below for Lab Range (LR) and Lab Product Range (LPR). LR indicates the spread in reported results among all of the labs for a given product while LPR allows for a comparison among the labs for the spread in results across the product set. A low LR value and a similar LPR for results would indicate agreement in results among the laboratories. The LR value indicates how large a difference might we expect in reported values from one lab to another. The LPR value indicates how similar or different the reported values distinguish the samples from one another.

Lab Range for a product (LR) = (labs' maximum mean – labs' minimum mean) / average of means of the three labs

Lab Range across products (LPR) = (lab's maximum mean – lab's minimum mean) / average of means for the lab for samples A–F.

RESULTS AND DISCUSSION

The study was conducted as a completely randomized design in a two-factor arrangement. The two major factors were: Products (with six levels defined by size or weight, making technique and tipping) and Laboratory (with three levels as 1, 2, 3). The products were characterized across 36 variables.

Physical parameters

The value in determining physical parameters is that these measures may indicate the natural variability of product manufacture. Some combinations of tobacco weight, cigar length, and cigar diameter are control parameters during manufacturing for most products. Pressure drop may be controlled/measured and is anticipated to impact smoke analytes' variability significantly.

Typically, the greater the variability in physical parameters, the greater the variability (% RSD and/or LPR) in smoking measurements and analytes. Smoke yields and variability correlate to tobacco weight; higher or more variable weight would likely result in more analyte or variability for an analyte, respectively. Pressure drop is a measure of relative resistance to airflow through the cigar column and is reported in units of mm of water. It is imperative to understand this parameter for machine smoking of cigars, given that airflow is established as a 'constant' under the smoking regime employed. Particulates' yield is generally inversely proportional to pressure drop; higher airflow resistance would result in an analyte's lower yield.

Each sample was tested for weight, length, diameter, and pressure drop (sometimes referred to as resistance to draw) (n = 20). Results collected for physical parameters from three different labs are displayed in Table 2. Data are presented as mean ± standard deviation with percent relative standard deviation (% RSD) as a parenthetical. Incidents of deviation included some cases where only one value was reported. For example, in the case of cigar weight, Lab 3 weighed all cigars together and divided by twenty. Additionally, Lab 3 did not report conditioned tobacco weight for samples A–D and only reported physical parameter data for one replicate of samples E and F. The range in nominal and reported values among the samples was quite broad, and the samples were easily distinguishable based on the significant differences in physical parameter measurements. For example, conditioned cigar weight among the samples ranged from less than 1.5 g/cigar to approximately 20 g/cigar. Diameter results ranged from less than 8 mm for Sample A to more than 20 mm for Sample F. Pressure drop results were inversely proportional to cigar length and diameter and ranged from approximately 270 mm H_2O for Sample A to 35 mm H_2O for Sample F.

Among the measured parameters in the study, physical parameters' replicates showed relatively low variability compared to tobacco and smoke results. See percent relative standard deviation (% RSD) calculations in Table 2. Of note, the typical % RSD for CORESTA Monitor cigarette weights is less than 1% (30) compared to as high as 10% (Sample C) observed for cigar samples in this study. Minimal variability was observed for length and diameter. On the other hand, pressure drop % RSD values and laboratory range (LR) values are as high as 43% and 33%, respectively. Figure 2 displays the mean and three times standard deviation for the samples' pressure drops. Sample A, with the highest pressure drop, has the lowest variability (~5%) and the lowest spread in reported results (10%). This is not surprising given that this sample type is the only design amenable to measurement with conventional testing equipment in use among the laboratories. Standardized equipment customized for cigar physical parameter testing is limited and cost-prohibitive. Thus, other cigars in the study present challenges to measuring pressure drop. While there is commercial, manual, or automated equipment available for cigarettes and cigars similar in diameter and length to cigarettes, larger cigars (Sample E and F), non-cylindrical cigars, and cigars with unusual shapes (Sample B) or mouthpieces (Sample D) must be measured with improvised, non-standardized equipment.

Despite the challenges of measuring physical parameters for a diverse sample set, the three laboratories were able to generate mean data that was similar between the labs. Not surprisingly, the average conditioned cigar weight (g/cigar) values were consistent over the cigar sample set as demonstrated by the low LR values for each sample. In addition, the range of conditioned cigar weight means observed for each laboratory, LPR, spanned comparable ranges 234% to 245%. The other physical parameter means for Samples A–F in Table 2 also demonstrate similar consistency between the three laboratories. Overall, the difference in LPR values for the laboratories ranged from 2 to 12 indicating a similar level of discernability among the labs for the physical parameters; one would anticipate similar results from any of the three labs for products of this divergent range in design.

Table 2. Summary of physical parameters results. Data are displayed as mean ± one standard deviation (% RSD); n = 20.

Lab	A	B	C	D	E	F	LPR [b]
Conditioned cigar weight (g/cigar)							
1	1.36 ± 0.02 (1)	2.69 ± 0.17 (6)	2.97 ± 0.23 (8)	3.91 ± 0.27 (7)	13.87 ± 1.24 (9)	18.05 ± 0.60 (3)	234%
2	1.37 ± 0.02 (2)	2.68 ± 0.10 (4)	3.04 ± 0.25 (8)	3.93 ± 0.27 (7)	13.58 ± 1.06 (8)	19.27 ± 1.14 (6)	245%
3	1.37 ± 0.02 (1)	2.72 ± 0.16 (6)	2.98 ± 0.28 (10)	3.92 ± 0.34 (9)	13.89	18.20	234%
LR[a]	1%	1%	2%	1%	2%	7%	
Conditioned tobacco weight (g/cigar)							
1	0.98 ± 0.02 (2)	2.19 ± 0.10 (5)	2.55 ± 0.22 (8)	2.22 ± 0.23 (11)	13.55 ± 1.25 (9)	17.62 ± 0.59 (3)	255%
2	0.97 ± 0.02 (2)	2.22 ± 0.08 (4)	2.59 ± 0.25 (10)	2.23 ± 0.26 (12)	13.39 ± 1.38 (10)	18.80 ± 1.18 (6)	266%
3	NR	NR	NR	NR	13.89	18.20	NC
LR	1%	1%	2%	0%	4%	6%	
Length (mm)							
1	98.3 ± 0.2 (0.2)	98.5 ± 1.2 (1.2)	113.7 ± 0.3 (0.3)	121.4 ± 0.5 (0.4)	127.0 ± 0.4 (0.3)	151.6 ± 0.5 (0.4)	45%
2	98.7 ± 0.2 (0.2)	102.5 ± 1.3 (1.3)	112.8 ± 0.2 (0.2)	121.5 ± 0.5 (0.4)	126.2 ± 0.6 (0.5)	152.2 ± 0.7 (0.5)	45%
3	98.9 ± 0.3 (0.3)	99.4 ± 0.8 (0.8)	103.2 ± 0.5 (0.5)	121.3 ± 0.5 (0.4)	120.7	141.1	37%
LR	1%	4%	10%	0%	5%	7%	
Diameter (mm)							
1	7.70 ± 0.02 (0.3)	9.93 ± 0.14 (1.5)	10.63 ± 0.04 (0.4)	11.35 ± 0.05 (0.4)	19.79 ± 0.44 (2.2)	20.97 ± 0.37 (1.8)	99%
2	7.82 ± 0.02 (0.3)	10.38 ± 0.21 (2.0)	10.67 ± 0.14 (1.3)	11.59 ± 0.06 (0.5)	20.07 ± 0.35 (1.7)	21.56 ± 0.27 (1.3)	100%
3	7.82 ± 0.06 (0.7)	11.17 ± 0.34 (3.1)	10.65 ± 0.18 (1.7)	11.17 ± 0.25 (2.3)	20.03	21.29	98%
LR	2%	12%	0%	4%	1%	3%	
Pressure drop (mm H_2O)							
1	260.7 ± 13.8 (5)	104.7 ± 29.1 (28)	127.3 ± 30.8 (24)	96.3 ± 41.5 (43)	37.4 ± 11.2 (30)	37.9 ± 11.6 (31)	202%
2	266.6 ± 17.0 (6)	87.0 ± 21.8 (25)	155.6 ± 50.7 (33)	85.7 ± 29.0 (34)	47.4 ± 14.3 (30)	33.0 ± 7.0 (21)	208%
3	287.6 ± 14.0 (5)	107.7 ± 26.8 (25)	134.2 ± 53.9 (40)	86.6 ± 28.4 (33)	52.3	35.9	214%
LR	10%	21%	20%	12%	33%	14%	

[a] LR: Lab Range = (labs' maximum mean – labs' minimum mean) / average of means the three labs;
[b] LPR: Lab Range across products = (lab's maximum mean – lab's minimum mean) / average of means for the lab for samples A–F;
< value indicates reported results had one or more values below the method limit of quantitation;
*includes at least one dataset with < values used in the calculation;
NR = not reported; NC = not calculated.

Control chart / reference information

Traditional cigarette testing incorporates the use of monitor or reference cigarettes and smokeless tobacco products that serve as positive controls and provide quality metrics for standardized analytical methods. They are useful for comparing data, in context, across studies, or among different laboratories in the same study. Key examples are University of Kentucky reference cigarettes and CORESTA monitor cigarettes. Each of these reference products can serve as a single positive control and an indicator of method variability within and among laboratories for all

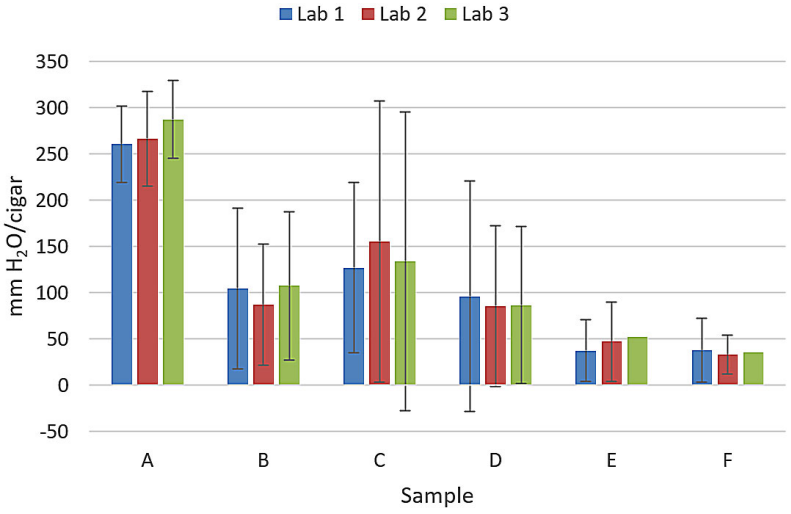

Figure 2. Lab comparison: Pressure drop average of 7 replicates for each sample from three different labs. Error bars, if shown, are 3 x standard deviation.

analytes of interest. The manufacture, design, and function of these reference products are similar to those of commercial cigarettes. Only recently have finished cigar reference products been made available for testing. While there is an ongoing FDA-sponsored project to establish a set of certified cigar reference products (31), limited characterization data for standardized cigar products, particularly among different laboratories and smoke constituents, is available.

In the absence of well-established standardized references for cigars, each laboratory in this study was encouraged to use its internal measures to ensure experimental robustness. In particular, established internal acceptance criteria for test batches were employed.

Tobacco analysis

Nicotine, ammonia, carbonyls, trace metals, BaP, and TSNAs (n = 7) were determined. Results shown in Table 3 are presented as dry weight basis mean ± standard deviation (% RSD) for before-grinding sampling. The spread in mean results was calculated and reported as LR in Table 3.

As with physical parameters measurements, the choice of samples included in the study afforded a wide range of measured values for LPR calculations. For example, nicotine ranged from approximately 8,000 µg/g to approximately 35,000 µg/g, and NNN showed a 21-fold range of results. BaP and crotonaldehyde were low (i.e., at or below LOQ) and indistinguishable among the samples supporting that these are not likely to be high valued measures for cigar tobacco characterization. The range in tobacco analyte levels is consistent with previous studies and is expected to be due to tobacco variety and growing and harvesting conditions (1).

In general, replicate analysis from each laboratory showed relatively low variability; % RSD were typically below 10%, indicating suitable method precision. Carbonyls were the exception, with variability as high as 31% for formaldehyde. This was expected given that these analytes are present at low, or less than LOQ levels, for the samples and tend to be problematic for analytical testing due to ubiquity and relative instability.

Quite a variation, as indicated by LR values in Table 3 and the deviation shown in Figure 3, was observed between labs for the tobacco analytes. This was particularly surprising for tobacco analytes with well-established methodology (2), such as nicotine and NNK with LRs as high as 34% and 247%, respectively. For nicotine, Lab 1 used a continuous flow analysis (CFA) method while labs 2 and 3 used chromatography. But a review of the data does not support a bias between Lab 1 and the other labs due to methodology; the differences do not consistently trend high or low for Lab 1. For the same batch of a product sample, one laboratory reported NNK as 4,388 ng/g while another reported 10,164 ng/g. The low % RSDs reported for a single batch of samples by each laboratory for the tobacco analytes support reasonable method precision. However, the relatively large LR values for most tobacco analytes indicate that the variation in results obtained between labs is more likely due to differences in methodology and inherent product variability. Differences in LPR values also lead one to question the source of differences in the span of means for the different laboratories. Although each individual cigar sample was made up of cigars from the same lot or at least from the same box, product variability appears to be inherent for cigars, especially long filler, hand rolled cigars. There have been considerable efforts towards method standardization, particularly by the CORESTA Tobacco and Tobacco Product Analysis (TTPA) Sub-Group. A review of recent proficiency and collaborative studies did not support that method differences were the likely concern with the spread in labs' results for this study. There was a recent product characterization study with the newly released cigar reference products that was useful in understanding the tobacco results in this study (32). Participating labs provided 8–13 data sets for a range of tobacco HPHCs and characterizing analytes. Results were used to estimate a true mean for each reference product, and *z*-scores were calculated for the labs. Generally, laboratories had positive *z*-scores (< 2), indicating agreement of results. Of note, based on ammonia, TSNA, and pH results, the 1C1 reference product data appeared to be bifurcated, and additional testing confirmed these findings.

Analyzing the TTPA study data in the context of this study was enlightening. The laboratory range of the TTPA data (calculated as the percent difference between the highest and lowest reported values) is consistent with the LR results for this study. Nicotine varied by as much as ~30%, and other analytes by 50–80%, excluding 1C1 comparisons. The bifurcated 1C1 results differed as much as 120% between the high and low reporting laboratories. There were sufficient participating labs to conclude that even with this spread in data, the labs' results were considered consistent based on *z*-scores. Also, the range in analyte results across the reference samples was similar to this study's diverse product set, and cigars of a similar design between the two studies (e.g., 1C2 reference product and Sample A) had similar results. This last point supports the value of the reference set to a broad product portfolio.

While we purposely selected products in this study from single lots of product, we have learned from LINDEGAARD's research even for samples produced from tobacco grown from single lots of seed and grown in the same locale, finished product cigar tobaccos may produce very different results (33). Arsenic, NNN, and nicotine varied by 379%, 115%, and 11%, respectively. LINDEGAARD's results are definitive confirmation of the inherent variability of the tobacco itself.

From the results of this study, taken in context with the work of other researchers and considering a long-term historical analysis of cigarette tobacco (34), it is clear that cigar characterization using tobacco constituent analysis will require ongoing analysis of reference products like the 'RT' or '1C' series to gain a grasp on the practical relevance of differences observed among data sets for the same product. For example, for a given analyte, 1,000 ng/g *vs.* 10,000 ng/g may be within the expected range of a given product.

Overall, the difference in LPR values for the laboratories ranged from 11 (nicotine) to 187 (arsenic) indicating differences in discernability among the labs for some of the

Table 3. Summary of tobacco testing results. Data are displayed as mean ± one standard deviation (% RSD); DWB = dry weight basis; n = 7.

Lab	A	B	C	D	E	F	LPR [b]
Nicotine DWB (µg/g)							
1	11065 ± 382 (3.5)	22419 ± 642 (2.9)	8336 ± 184 (2.2)	9742 ± 391 (4.0)	23666 ± 938 (4.0)	29138 ± 1042 (3.6)	120%
2	12858 ± 596 (4.6)	18114 ± 928 (5.1)	7832 ± 393 (5.0)	11996 ± 604 (5.0)	31864 ± 2633 (8.3)	28475 ± 4084 (14.3)	130%
3	14797 ± 622 (4.2)	23685 ± 89 (0.4)	8126 ± 60 (0.7)	13734 ± 69 (0.5)	26030 ± 253 (1.0)	34491 ± 264 (0.8)	131%
LR [a]	29%	26%	6%	34%	30%	20%	
Ammonia DWB (µg/g)							
1	2771 ± 41 (1.5)	4769 ± 85 (1.8)	2116 ± 35 (1.7)	1616 ± 14 (0.9)	6990 ± 124 (1.8)	8593 ± 85 (1.0)	156%
2	3112 ± 73 (2.4)	4608 ± 71 (1.5)	2344 ± 25 (1.1)	1929 ± 37 (1.9)	8048 ± 178 (2.2)	8281 ± 233 (2.8)	135%
3	3584 ± 144 (4.0)	5497 ± 140 (2.6)	2529 ± 56 (2.2)	1841 ± 23 (1.2)	8641 ± 213 (2.5)	9745 ± 107 (1.1)	149%
LR	26%	18%	18%	17%	21%	16%	
Acetaldehyde DWB (µg/g)							
1	1.17 ± 0.16 (14)	0.21 ± 0.04 (19)	0.97 ± 0.03 (3)	0.29 ± 0.05 (16)	0.65 ± 0.11 (17)	1.28 ± 0.14 (11)	140%
2	2.23 ± 0.12 (6)	< 0.10	2.07 ± 0.30 (15)	0.81 ± 0.08 (10)	0.75 ± 0.02 (3)	1.28 ± 0.22 (17)	NC
3	0.50 ± 0.01 (2)	0.22 ± 0.02 (9)	1.11 ± 0.07 (6)	< 0.10	0.33 ± 0.03 (8)	0.61 ± 0.06 (10)	161%
LR	133%	5%	80%	95%	73%	63%	
Crotonaldehyde DWB (µg/g)							
1	< 0.040	< 0.041	< 0.041	< 0.039	< 0.042	< 0.042	NA
2	< 0.050	< 0.050	< 0.050	< 0.050	< 0.050	< 0.050	NA
3	< 0.050	< 0.050	< 0.050	< 0.050	< 0.009	< 0.009	NA
LR	NA	NA	NA	NA	NA	NA	
Formaldehyde DWB (µg/g)							
1	1.22 ± 0.17 (14)	0.51 ± 0.05 (10)	0.71 ± 0.03 (4)	3.33 ± 0.45 (14)	1.19 ± 0.18 (15)	1.39 ± 0.18 (13)	203%
2	1.03 ± 0.08 (7)	0.46 ± 0.09 (20)	1.14 ± 0.11 (10)	2.32 ± 0.11 (5)	0.58 ± 0.01 (1)	0.98 ± 0.12 (12)	171%
3	0.83 ± 0.07 (8)	0.26 ± 0.08 (31)	0.78 ± 0.09 (12)	1.21 ± 0.08 (7)	0.69 ± 0.07 (11)	0.52 ± 0.08 (15)	133%
LR	38%	61%	49%	93%	74%	90%	
Arsenic DWB (ng/g)							
1	99 ± 6 (6)	146 ± 11 (8)	2342 ± 554 (24)	2080 ± 162 (8)	111 ± 4 (3)	133 ± 6 (5)	274%
2	140 ± 15 (10)	120 ± 11 (9)	216 ± 17 (8)	281 ± 30 (11)	157 ± 18 (12)	189 ± 16 (8)	88%
3	129 ± 10 (7)	211 ± 16 (8)	1028 ± 125 (12)	265 ± 14 (5)	188 ± 19 (10)	140 ± 10 (7)	275%
LR	33%	57%	178%	207%	51%	36%	
Cadmium DWB (ng/g)							
1	1078 ± 40 (4)	1164 ± 45 (4)	1173 ± 134 (11)	1326 ± 29 (2)	527 ± 16 (3)	722 ± 25 (3)	80%
2	1414 ± 88 (6)	1381 ± 109 (8)	1316 ± 84 (6)	1622 ± 98 (6)	669 ± 51 (8)	328 ± 81 (25)	115%
3	1273 ± 88 (7)	1416 ± 34 (2)	1312 ± 18 (1)	1770 ± 15 (1)	1216 ± 69 (6)	769 ± 15 (2)	77%
LR	27%	19%	11%	28%	86%	73%	
Benzo(a)pyrene DWB (ng/g)							
1	4.33 ± 0.31 (7)	4.63 ± 0.39 (8)	4.57 ± 0.23 (5)	4.14 ± 0.25 (6)	3.86 ± 0.25 (7)	1.85 ± 0.14 (8)	71%
2	5.38 ± 0.35 (7)	5.85 ± 0.26 (5)	5.63 ± 0.62 (11)	3.19 ± 0.27 (9)	< 2.19	< 2.00	NC
3	3.72 ± 0.81 (22)	4.10 ± 0.25 (6)	< 0.10	3.20 ± 0.31 (10)	3.05 ± 0.33 (11)	2.11 ± 0.25 (12)	NC
LR	37%	36%	21%	27%	23%	13%	
NNK DWB (ng/g)							
1	4682 ± 149 (3)	661 ± 43 (6)	4388 ± 137 (3)	445 ± 19 (4)	687 ± 17 (2)	164 ± 18 (11)	246%
2	2701 ± 475 (18)	477 ± 36(8)	8938 ± 138(2)	341 ± 11(3)	< 100	2425 ± 132(6)	NC
3	2987 ± 162(5)	606 ± 24(4)	10164 ± 426(4)	472 ± 28(6)	1245 ± 255(21)	163 ± 22(13)	384%
LR	57%	32%	74%	31%	58%	247%	
NNN DWB (ng/g)							
1	5326 ± 295(5.5)	3012 ± 155(5.1)	10016 ± 495(4.9)	1850 ± 127(6.9)	2186 ± 60(2.8)	2050 ± 87(4.2)	200%
2	4896 ± 249(5.1)	2674 ± 241(9.0)	16749 ± 187(1.1)	1625 ± 6(0.4)	1244 ± 85(6.8)	5173 ± 239(4.6)	287%
3	5821 ± 224(3.8)	3632 ± 92(2.5)	26566 ± 752(2.8)	3140 ± 160(5.1)	3795 ± 347(9.1)	1993 ± 46(2.3)	328%
LR	17%	31%	93%	69%	106%	104%	

[a] LR: Lab Range = (labs' maximum mean – labs' minimum mean) / average of means the three labs;
[b] LPR: Lab Range across products = (lab's maximum mean – lab's minimum mean) / average of means for the lab for samples A–F;
< value indicates reported results had one or more values below the method limit of quantitation;
* includes at least one dataset with < values used in the calculation;
NC = not calculated; NA = Not applicable.

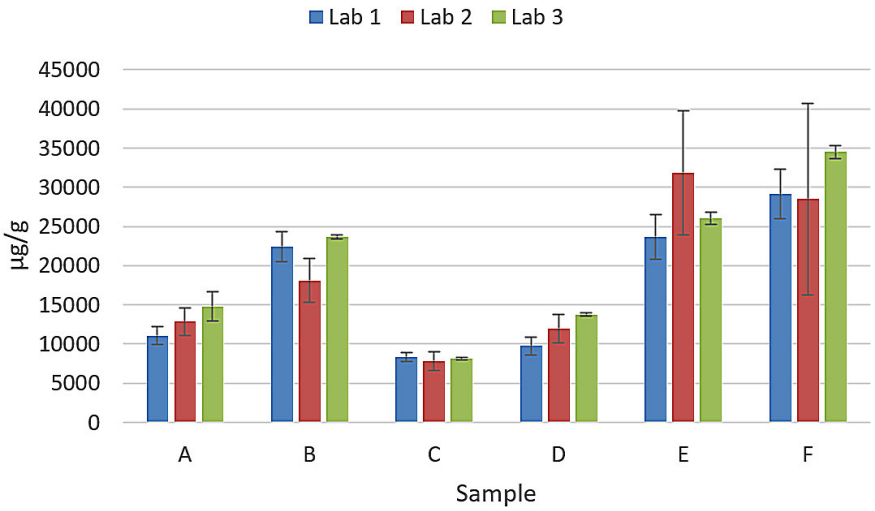

Figure 3. Lab comparison: Tobacco nicotine average of 7 replicates shown for each sample from three different labs. Error bars are 3 x standard deviation.

tobacco analytes, either due to product variability, even from the same lot, method differences among the labs, or a combination of both.

Preparation for smoking

Requirements for preparing cigars for smoking have a foundation in cigarette preparation requirements with differences attributable to the unique features of cigars. Cigars must be stored under specified temperature and humidity to allow for stabilized weight. The conditioning period is set to a minimum of 72 hours with no maximum time specified. Due to size, some cigars' conditioning may require as long as multiple weeks to reach weight stabilization. Cigars with volatile additives may be unable to weight stabilize, and an analyst must use judgment when to discontinue the equilibration session. In this study, one of the labs reported issues with weight stabilization for the highly flavored sample with a request for instructions. Cigars with a closed mouth-end must be cut prior to smoking. Cutting distance is specified but may need judgment in cases where the mouth-end is not symmetrical or where a wrapper is held on by a glue spot along the cutting line. Additionally, cutting tools for analytical purposes are not standardized. These examples of judgment may contribute to analytical variability but to a lesser extent than product variability and the variability due to the use of non-standardized methods.

Smoking - HPHCs

Machine smoking of combustible tobacco products under standardized regimes is for comparative purposes and is not intended to represent the range of consumer smoking behaviors. Thus, it is important to employ standardized equipment, reference products, and methodology to allow the comparison of different products under a common set of controlled conditions. For conventional cigarette products, this standardization process began at least as early as the 1960s and is ongoing through organizations such as CORESTA and ISO Technical Committee ISO/TC 126, among others (35). There are multiple standardized smoking regimes (36, 37) as well as several reference and monitor products designed to support conventional machine-made cigarettes.

Regarding cigar smoking, the product category is relatively broad for design which has created a challenge for standardizing smoking parameters. Industry experts designed a regime based on maintaining a constant puff velocity to allow comparison of products with different diameters (< 8 mm to > 25 mm) and to minimize self-extinguishment during machine smoking (38). The machine smoking parameters employed in this study are shown in Table 4.

Table 4. Employed machine smoking parameters.

Parameter	Cigar diameter ≤ 12.0 mm	Cigar diameter > 12.0 mm
Puff volume (mL)	20	0.139 × (diameter) ^{2}
Puff frequency (s)	40	40
Puff duration (s)	1.5	1.5

Smoking - TNCO

The methods for determination of TNCO ("tar", nicotine, carbon monoxide) are the only smoking methods for cigars to date to be regularly evaluated in lab-to-lab comparisons. The methodology has been used since 2005, with at least 16 completed lab comparison studies to establish and evaluate reproducibility and repeatability (*r* and *R*) values. Thus, TNCO should be the best-case scenario for smoking analytes for a lab-to-lab (LR) comparison of smoking and smoke constituents.

TNCO results are shown in Table 5 as mean ± standard deviation (% RSD) of 20 replicates for puff count, CO, nicotine, "tar", TPM, and water where a replicate is a pad/port count without regard to the number of cigars smoked per pad/port. LR values indicate the range between

Table 5. Summary results of puff count, TPM, "tar", nicotine, water, and CO. Data are displayed as mean ± one standard deviation (%RSD); n = 20;.

Lab	A	B	C	D	E	F	LPR [b]
Puff count (per cigar)							
1	21 ± 1 (4)	54 ± 10 (18)	45 ± 3 (7)	37 ± 3 (9)	92 ± 14 (16)	201 ± 59 (29)	240%
2	22 ± 1 (6)	57 ± 10 (18)	42 ± 3 (7)	50 ± 7 (15)	103 ± 13 (12)	155 ± 18 (12)	186%
3	20 ± 1 (7)	48 ± 7 (14)	41 ± 4 (9)	37 ± 5 (13)	155 ± 25 (16)	199 ± 32 (16)	215%
LR [a]	10%	17%	9%	31%	54%	25%	
"Tar" (mg/cigar)							
1	22 ± 2 (10)	37 ± 11 (30)	49 ± 5 (11)	31 ± 9 (30)	92 ± 47 (51)	138 ± 55 (40)	189%
2	25 ± 3 (13)	51 ± 8 (16)	50 ± 5 (11)	61 ± 8 (13)	138 ± 26 (19)	286 ± 47 (16)	256%
3	19 ± 3 (17)	45 ± 12 (25)	49 ± 8 (17)	39 ± 9 (22)	171 ± 41 (24)	291 ± 47 (16)	266%
LR	27%	32%	2%	69%	59%	64%	
Nicotine (mg/cigar)							
1	0.95 ± 0.11 (11)	2.26 ± 0.77 (34)	1.45 ± 0.41 (28)	1.08 ± 0.45 (41)	5.62 ± 3.45 (61)	22.24 ± 13.06 (59)	380%
2	1.16 ± 0.11 (9)	3.22 ± 0.49 (15)	1.51 ± 0.18 (12)	2.26 ± 0.30 (13)	9.91 ± 3.46 (35)	13.19 ± 5.69 (43)	231%
3	0.86 ± 0.09 (10)	3.33 ± 0.71 (21)	1.42 ± 0.25 (18)	1.49 ± 0.44 (30)	9.86 ± 4.22 (43)	25.70 ± 7.51 (29)	349%
LR	30%	36%	6%	73%	51%	61%	
Carbon monoxide (mg/cigar)							
1	28 ± 3 (11.0)	65 ± 18 (28.0)	69 ± 43 (62.0)	58 ± 17 (30.0)	< 359.8	553 ± 311 (56.0)	340% [c]
2	30 ± 5 (17.0)	70 ± 12 (18.0)	72 ± 21 (30.0)	78 ± 27 (35.0)	446 ± 172 (39.0)	751 ± 110 (15.0)	299%
3	26 ± 3 (13.0)	67 ± 13 (19.0)	93 ± 10 (10.0)	57 ± 8 (14.0)	404 ± 60 (15.0)	732 ± 69 (9.0)	307%
LR	14%	7%	31%	33%	21% [a]	29%	
Water (mg/cigar)							
1	< 0.6	< 2.2	1.9 ± 0.5 (25)	< 0.8	< 88.0	< 309.8	NC
2	0.8 ± 0.4 (49)	3.7 ± 1.6 (44)	2.6 ± 1.7 (66)	8.4 ± 2.7 (33)	96.9 ± 35.5 (37)	166.5 ± 53.6 (32)	356%
3	0.1 ± 0.2 (206)	0.7 ± 1.0 (141)	0.8 ± 0.8 (101)	1.0 ± 1.0 (106)	88.8 ± 34.8 (39)	261.9 ± 97.4 (37)	445%
LR	140% [c]	136% [c]	102%	224% [c]	10% [c]	58% [c]	
TPM (mg/cigar)							
1	23 ± 2 (11)	42 ± 13 (32)	52 ± 6 (11)	32 ± 10 (31)	185 ± 102 (55)	470 ± 261 (56)	334%
2	27 ± 3 (12)	58 ± 9 (15)	54 ± 6 (11)	71 ± 10 (14)	245 ± 40 (16)	466 ± 78 (17)	286%
3	19 ± 3 (17)	50 ± 12 (25)	52 ± 8 (16)	42 ± 10 (23)	269 ± 72 (27)	579 ± 111 (19)	332%
LR	35%	32%	4%	81%	36%	22%	

[a] LR: Lab Range = (labs' maximum mean – labs' minimum mean) / average of means the three labs;
[b] LPR: Lab Range across products = (lab's maximum mean – lab's minimum mean) / average of means for the lab for samples A–F;
[c] includes at least one dataset with < values used in the calculation;
< value indicates reported results had one or more values below the method limit of quantitation;
NR = not reported; NC = not calculated.

the different laboratories' results. As expected based on physical parameters (e.g., tobacco weight), the range (LPR) in puff count among the products was incredibly wide at 20–200 puffs/cigar. The spread in values among the other measures is similar to the puff count trend. The spread in values among the other measures follows the trend of increasing analyte delivery with increasing puff count. The difference in LPR values for the laboratories ranged from 40 to 150 indicating less consistency in results among the laboratories for the products compared to physical parameter measures.

The variability in replicates for the measures is much higher than observed for tobacco measures. This would be expected due to increased complexity for smoking: the addition of a wide range of sample preparation variables related to smoking and the, typically, more complex clean-up and chromatographic analysis of the resulting extracts. Sample A would be considered the easiest to smoke based on size, shape, and relative yields, and it tended to have the lowest % RSD among the samples. The % RSD for water was strikingly high, particularly for Lab 3. Water is a problematic analyte due to its ubiquitous nature, and is formed during combustion processes. The water yields for cigars are relatively high; in our work, we have seen water breakthrough to the smoking machine tubing and CO collection bags.

Sample D, which had a non-cylindrical and tipped mouth-end, had the highest LR in results at 33%–224% for TNCO and water. This was anticipated due to the non-cylindrical mouth-end being more challenging to seal in the cigar holder and is likely due to differences in handling practices and management of the cigar-cigar holder interface. In practice, laboratories use the commercially available holders described in CRM 64 with adaptations for the mouthpiece or custom-made holders of unknown design for samples with non-cylindrical mouth-ends. Lack of standardization and potentially lack of experience with such products may lead to high LR values.

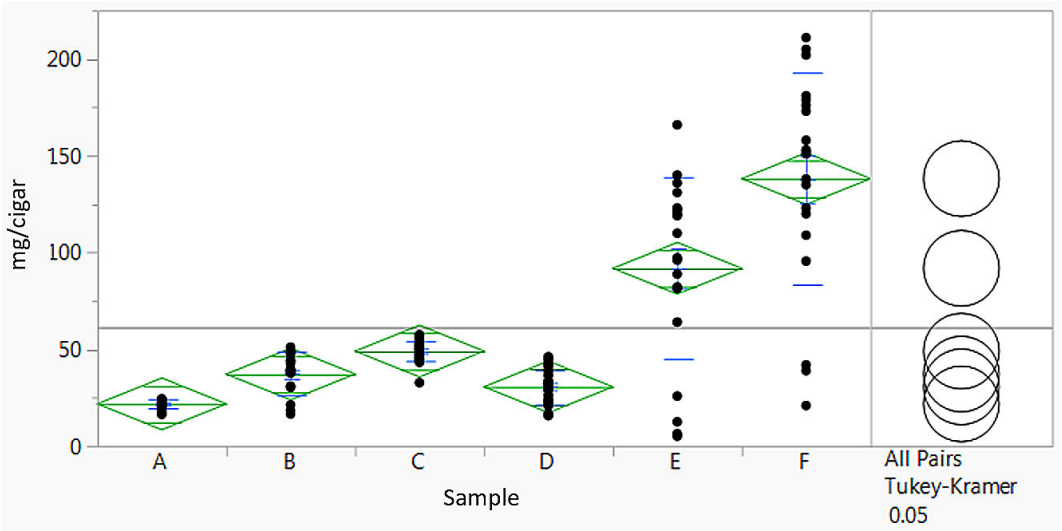

Figure 4. Product comparison: "Tar" yields. Data shown for a single laboratory (Lab 1), n=20.

Given the relatively high replicate variability and LR results, a particular question for TNCO results from the study is: Given the range of design features, are the samples distinguishable from each other based on TNCO results? This was not the case in this study. Figure 4 shows an ANOVA comparison for the samples' "tar" using the data from one laboratory. The vertical span of the diamonds represents the 95% confidence interval for the mean, and the line near the center represents the mean "tar" value for each sample. Only Samples E and F are statistically different from the other samples when compared pairwise using Tukey-Kramer analysis. It is notable that Samples E and F show a very large spread in replicate values. Reproducibility and repeatability results from the 16th annual TNCO collaborative study conducted by CORESTA support the lack of statistical significance. %R values determined from the results of 7 laboratories (n = 5) ranged from ~30% to 70% for products similar to Sample C in this study (39).

Research has shown that laboratories with significant experience testing relatively less-challenging cigars have demonstrated improvement in data agreement when using standardized methodology for cigar smoking. For example, Figure 5 shows a trending improvement in %R values across 11 years of collaborative TNCO testing (40) but also highlights relatively low participation compared to collaborative testing of tobacco and other products.

Interestingly, prior to the release of cigar reference products, the TNCO collaborative studies were conducted using cigarette monitor test pieces as a study control of sorts. As displayed in Figure 6, with means of a similar magnitude, coefficient of variability (CV) of *r* and *R* values between a non-intense cigarette regime and the cigar-smoking regime are similar (40, 41). This is encouraging regarding the cigar-smoking method's capabilities but points to the relative difficulty for testing and inherent variability of some samples as the prominent cause of higher variability among the TNCO data in this study.

A drive toward simplifying and harmonizing techniques and acknowledging that some cigar designs are not particularly amenable to machine smoking may be warranted for product characterization within and between lab-

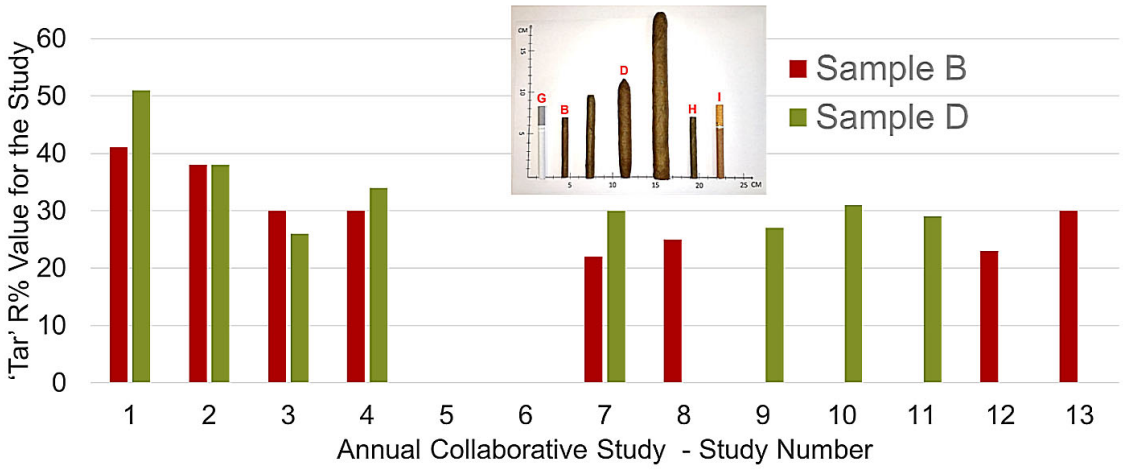

Figure 5. TNCO Collaborative Studies: %R values for "tar" from 2007 to 2019 (n=5; laboratory count, typically <12).

CM8 – 2017 Cigar Collaborative Study

	Global Mean	CVr	CVR
Weight	953.7	1.7%	2.2%
TPM	15.16	15.2%	26.3%
Water	0.87	52.3%	100.9%
Nicotine	1.427	11.5%	23.1%
NFDPM	12.87	16.3%	29.3%
CO	9.73	13.9%	37.5%

Number of Labs = 11
n= 5 replicates of 8 cigarettes per replicate, 2 cigarettes/pad (4 pads)

Smoking Method = CRM 64/65

CM8 - 2017 Cigarette Collaborative Study

	Global Mean	CVr	CVR
Weight	959.7	0.45%	0.63%
TPM	17.370	2.2%	3.9%
Water	1.820	7.7%	22.3%
Nicotine	1.672	2.0%	5.4%
NFDPM	13.952	2.2%	4.7%
CO	13.208	2.6%	5.3%

Number of Labs = 34, some with multiple data sets
n= 5 replicates of 5 cigs/pad (4 pads) or 20 cigs/pad (1 pad),depending on pad size
Smoking Method = ISO 3308

Figure 6. TNCO Collaborative studies: Cigar (40) *vs.* cigarette (41) regime for a cigarette monitor test piece where global mean is based on data from all laboratories in the study.

oratories. As with analysis and interpretation of tobacco, considering a given dataset in context is critical to decision making.

Smoke analysis - other constituents

Methods in use for smoke constituents are typically based on established cigarette methods with necessary modifications for cigars typically due to increased yield and smoke times. These modifications have been made in isolation rather than through collaborative studies. Thus, it is likely that there are many unknown but relevant differences between different labs' methods.

Results for ammonia, and selected carbonyls, PAAs, PAHs, TSNAs, and VOC are shown in Table 6 as mean ± standard deviation (% RSD) of seven replicates for selected smoke constituents along with LR values for the analytes. For constituent evaluation, data are consistently high for % RSD and LR values. The spread in values between the products (LPR) is approximately 10- to 30-fold.

Ammonia and carbonyl results are particularly interesting in the dataset. In addition to high replicate variability for ammonia, there was no consistency between the three labs. For example, Sample D results ranged from < LOQ to over 100 µg/cigar between the labs' datasets. Additionally, the ammonia yields for samples E and F are significantly higher than those for samples A–D, suggesting possible ammonia formation during smoking due to the difficulty and length of time required to machine-smoke larger cigars. Research by PREPELITSKAYA *et al.* confirms the likelihood of artificial generation of ammonia under certain experimental conditions and the breakdown of ammonia under other conditions. They also confirmed that typical cigarette methodology is not applicable to all cigars (42, 43). Given the results in this study and the background research of PREPELITSKAYA, the reported ammonia results here would be considered as suspect.

It was anticipated that carbonyls, formaldehyde in particular, would be the most problematic for methods development and testing of cigars. Carbonyl methods involve time-sensitive derivatization that requires optimization of pH and the total amount of derivatizing agent. A direct adaptation of a cigarette method is unlikely to be successful for cigars due to smoking times that may go into hours, compared to approximately 10 minutes for cigarettes. Besides variability and range issues across the labs' results, the trend for each lab across the samples is notably different for formaldehyde, potentially elucidating methodological issues akin to those suspected for ammonia. As displayed in Figure 7, Lab 2 results trend up across the entire sample range (A–F). At the same time, Lab 1 and Lab 3 appear to have a similar trend in data for A–D but differentiate from Lab 2 for E and F. Crotonaldehyde results for Sample B appear anomalous for Lab 2 in particular, but with no assignable cause for the striking difference in the data reported. There is no consistency among the acetaldehyde or acrolein data reported by the three labs. The difference in LPR values for the laboratories ranged from 47 to 143 indicating less consistency in results among the laboratories for the products compared to physical parameter measures. This information and the relatively high % RSD and LR values for the smoke constituents, reinforce continued methods refinement and use of reference products to aid data analysis and comparison between studies.

Study limitations

The study's objective was an initial assessment of product range and variability and laboratory range (i.e., variability) for single batch analysis of selected cigars of typical design. Using only one batch of product does not allow for evaluating product batch-to-batch variability. Research akin to the CORESTA Cigarette Variability Task Force (CVAR) study for cigarette batch-to-batch variability (44) to understand cigar product variability, though likely much more challenging than similar undertakings for other product categories, is in progress (45). The number of laboratories in the study does not allow for the determination of *z*-scores, outliers, or repeatability and reproducibility values. It may or may not be appropriate to compare tobacco nicotine (total alkaloids) and smoke nicotine (nicotine) due to differences in analytes measured.

Table 6. Summary of smoking results. Data are displayed as mean ± one standard deviation (%RSD); n = 7;.

Lab	A	B	C	D	E	F	LPR [b]
Ammonia (µg/cigar)							
1	28 ± 7 (27)	60 ± 53 (87)	107 ± 129 (121)	104 ± 88 (85)	1467 ± 1167 (80)	5987 ± 3397 (57)	461%
2	43 ± 12 (28)	43 ± 9 (21)	62 ± 5 (9)	68 ± 13 (19)	1101 ± 215 (20)	2636 ± 1581 (60)	394%
3	< 30.0	< 30.2	41 ± 7 (16)	< 32.9	1503 ± 996 (66)	5417 ± 2979 (55)	NC
LR [a]	45%	67%	94%	104%	30%	72%	
Acetaldehyde (µg/cigar)							
1	700 ± 133 (19)	955 ± 286 (30)	1721 ± 357 (21)	1769 ± 454 (26)	4669 ± 1925 (41)	5011 ± 1134 (23)	174%
2	760 ± 67 (9)	1334 ± 286 (21)	1653 ± 263 (16)	1747 ± 204 (12)	3257 ± 726 (22)	5520 ± 1082 (20)	200%
3	1042 ± 73 (7)	1906 ± 121 (6)	2525 ± 199 (8)	2251 ± 206 (9)	3892 ± 728 (19)	4503 ± 758 (17)	129%
LR	41%	68%	44%	26%	36%	20%	
Acrolein (µg/cigar)							
1	27 ± 6 (24)	11 ± 3 (30)	26 ± 9 (35)	33 ± 11 (33)	63 ± 12 (19)	99 ± 16 (16)	204%
2	36 ± 4 (10)	29 ± 3 (11)	47 ± 10 (22)	47 ± 10 (21)	67 ± 18 (27)	92 ± 19 (20)	119%
3	40 ± 3 (8)	26 ± 2 (8)	51 ± 5 (9)	51 ± 5 (9)	28 ± 3 (11)	63 ± 9 (14)	86%
LR	38%	82%	60%	41%	74%	43%	
Crotonaldehyde (µg/cigar)							
1	6 ± 1 (21)	9 ± 3 (31)	42 ± 16 (39)	38 ± 11 (28)	61 ± 24 (39)	77 ± 19 (25)	183%
2	15 ± 2 (15)	195 ± 38 (20)	44 ± 8 (19)	49 ± 8 (16)	49 ± 10 (20)	68 ± 23 (34)	257%
3	11 ± 1 (7)	20 ± 2 (10)	40 ± 5 (12)	44 ± 3 (7)	20 ± 5 (26)	27 ± 7 (27)	122%
LR	84%	249%	10%	25%	95%	87%	
Formaldehyde (µg/cigar)							
1	11 ± 2 (18)	8 ± 3 (36)	11 ± 3 (26)	15 ± 4 (29)	8 ± 3 (33)	9 ± 3 (33)	68%
2	8 ± 2 (26)	9 ± 2 (22)	14 ± 3 (21)	15 ± 5 (33)	20 ± 3 (16)	24 ± 5 (23)	107%
3	7 ± 1 (11)	7 ± 1 (17)	13 ± 3 (22)	21 ± 3 (16)	9 ± 1 (13)	16 ± 3 (21)	115%
LR	46%	25%	24%	35%	97%	92%	
4-Aminobiphenyl (ng/cigar)							
1	4 ± 1 (17)	9 ± 2 (18)	15 ± 1 (8)	5 ± 3 (65)	41 ± 11 (28)	50 ± 10 (20)	223%
2	2 ± 0 (14)	6 ± 2 (27)	12 ± 1 (11)	8 ± 2 (27)	10 ± 4 (44)	17 ± 5 (28)	164%
3	3 ± 0 (9)	7 ± 2 (33)	13 ± 2 (15)	5 ± 2 (36)	19 ± 3 (15)	38 ± 7 (17)	247%
LR	67%	41%	23%	50%	133%	94%	
1-Aminonaphthalene (ng/cigar)							
1	53 ± 8 (15)	90 ± 18 (20)	137 ± 13 (10)	55 ± 38 (69)	424 ± 104 (24)	518 ± 124 (24)	218%
2	86 ± 7 (9)	118 ± 31 (27)	155 ± 21 (14)	150 ± 30 (20)	131 ± 54 (41)	193 ± 51 (26)	77%
3	39 ± 8 (20)	77 ± 34 (44)	111 ± 20 (18)	55 ± 23 (42)	170 ± 39 (23)	308 ± 45 (15)	212%
LR	79%	43%	33%	110%	121%	96%	
2-Aminonaphthalene (ng/cigar)							
1	35 ± 5 (14)	48 ± 8 (18)	78 ± 8 (10)	35 ± 23 (65)	221 ± 55 (25)	270 ± 65 (24)	205%
2	39 ± 2 (4)	43 ± 9 (21)	67 ± 5 (8)	68 ± 14 (20)	52 ± 21 (40)	76 ± 24 (32)	64%
3	26 ± 4 (16)	38 ± 15 (38)	60 ± 11 (19)	32 ± 13 (42)	88 ± 19 (21)	168 ± 38 (23)	207%
LR	39%	23%	26%	80%	140%	113%	
Benzo(a)pyrene (ng/cigar)							
1	11 ± 5 (48)	23 ± 9 (42)	74 ± 12 (15)	32 ± 19 (58)	97 ± 35 (36)	155 ± 30 (19)	220%
2	26 ± 4 (14)	39 ± 7 (19)	89 ± 11 (12)	85 ± 17 (20)	99 ± 19 (20)	137 ± 25 (19)	140%
3	19 ± 2 (11)	30 ± 12 (42)	68 ± 8 (11)	63 ± 12 (19)	78 ± 20 (26)	113 ± 47 (42)	152%
LR	80%	52%	27%	88%	23%	31%	
NNK (ng/cigar)							
1	433 ± 79 (18)	135 ± 36 (27)	689 ± 216 (31)	166 ± 82 (50)	421 ± 213 (51)	380 ± 268 (71)	149%
2	1286 ± 246 (19)	502 ± 111 (22)	4299 ± 682 (16)	574 ± 91 (16)	1707 ± 2358 (138)	529 ± 387 (73)	256%
3	419 ± 37 (9)	213 ± 77 (36)	1809 ± 327 (18)	152 ± 58 (38)	1266 ± 1492 (118)	255 ± 77 (30)	242%
LR	122%	130%	159%	142%	114%	71%	
NNN (ng/cigar)							
1	350 ± 64 (18)	240 ± 90 (37)	838 ± 184 (22)	224 ± 92 (41)	1303 ± 1025(79)	2364 ± 766 (32)	241%
2	1177 ± 73 (6)	1030 ± 215 (21)	6313 ± 1008 (16)	935 ± 96 (10)	2286 ± 1399 (61)	1989 ± 647 (33)	235%
3	402 ± 20 (5)	517 ± 228 (44)	3205 ± 482 (15)	322 ± 112 (35)	1755 ± 883 (50)	3370 ± 1597 (47)	191%
LR	129%	133%	159%	144%	55%	54%	

Table 6. contd.

Lab	A	B	C	D	E	F	LPR [b]
Acrylonitrile (µg/cigar)							
1	12 ± 3 (28)	55 ± 12 (21)	72 ± 15 (21)	30 ± 16 (54)	302 ± 56 (19)	473 ± 90 (19)	293%
2	17 ± 5 (27)	63 ± 11 (18)	81 ± 7 (8)	57 ± 17 (29)	112 ± 25 (23)	184 ± 37 (20)	195%
3	16 ± 1 (7)	65 ± 19 (29)	90 ± 12 (13)	51 ± 3 (7)	241 ± 36 (15)	488 ± 79 (16)	298%
LR	33%	16%	22%	59%	87%	80%	
Benzene (µg/cigar)							
1	58 ± 9 (16)	248 ± 75 (30)	280 ± 46 (16)	152 ± 72 (48)	1313 ± 144 (11)	1634 ± 144 (9)	257%
2	81 ± 19 (24)	320 ± 52 (16)	356 ± 29 (8)	305 ± 80 (26)	699 ± 97 (14)	812 ± 168 (21)	170%
3	71 ± 4 (6)	318 ± 81 (25)	364 ± 52 (14)	231 ± 20 (9)	1250 ± 154 (12)	2021 ± 204 (10)	275%
LR	33%	24%	25%	67%	56%	81%	
1,3-Butadiene (µg/cigar)							
1	56 ± 4 (7)	185 ± 30 (16)	235 ± 46 (20)	116 ± 50 (43)	974 ± 304 (31)	974 ± 279 (29)	217%
2	207 ± 49 (24)	600 ± 130 (22)	757 ± 79 (10)	515 ± 131 (25)	1445 ± 724 (50)	1172 ± 233 (20)	158%
3	93 ± 5 (6)	323 ± 97 (30)	388 ± 37 (9)	245 ± 18 (7)	633 ± 124 (20)	1033 ± 158 (15)	208%
LR	127%	112%	113%	137%	80%	19%	
Isoprene (µg/cigar)							
1	527 ± 90 (17)	2019 ± 410 (20)	1897 ± 333 (18)	1001 ± 332 (33)	8714 ± 1988 (23)	11006 ± 3810 (35)	250%
2	736 ± 146 (20)	2027 ± 447 (22)	1864 ± 203 (11)	1739 ± 377 (22)	4360 ± 1348 (31)	4893 ± 1186 (24)	160%
3	630 ± 54 (9)	2253 ± 717 (32)	1898 ± 156 (8)	1490 ± 98 (7)	7131 ± 1804 (25)	14393 ± 2265 (16)	297%
LR	33%	11%	2%	52%	65%	94%	
Toluene (µg/cigar)							
1	84 ± 25 (29)	391 ± 93 (24)	407 ± 93 (23)	230 ± 93 (40)	2700 ± 413 (15)	3705 ± 642 (17)	289%
2	131 ± 33 (26)	544 ± 78 (14)	603 ± 70 (12)	575 ± 149 (26)	1273 ± 250 (20)	1823 ± 316 (17)	205%
3	128 ± 9 (7)	621 ± 140 (22)	732 ± 116 (16)	458 ± 47 (10)	2706 ± 301 (11)	5041 ± 536 (11)	304%
LR	41%	44%	56%	82%	64%	91%	

[a] LR: Lab Range = (labs' maximum mean – labs' minimum mean) / average of means the three labs;
[b] LPR: Lab Range across products = (lab's maximum mean – lab's minimum mean) / average of means for the lab for samples A–F;
[c] includes at least one dataset with < values used in the calculation;
< value indicates reported results had one or more values below the method limit of quantitation;
NR = not reported; NC = not calculated.

CONCLUSIONS

This study aimed to characterize cigars selected based on a range of design features (e.g., tobacco weight 1–20 g/cigar) and evaluate the range in magnitude of the measures (LPR) and the analytical variability (% RSD) for selected HPHCs among replicates and across multiple laboratories (LR). A conventional smoking regime established for cigar TNCO was used for smoking, and analysis for tobacco and smoke HPHCs was conducted using non-standardized methodology in routine use in each laboratory in the study. In all cases, LPR, % RSD, and LR

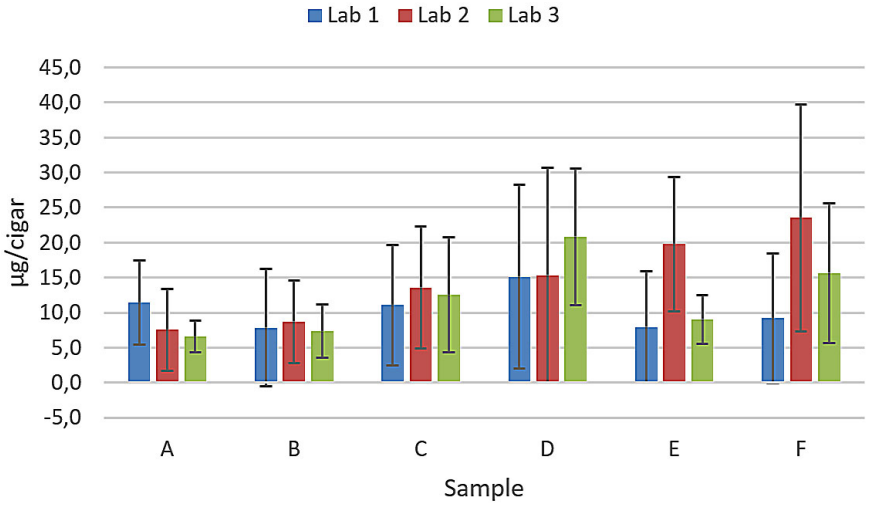

Figure 7. Lab comparison: Smoke formaldehyde average of 7 replicates shown for each sample from three different labs. Error bars are 3 x standard deviation.

were relatively large compared to cigarette products. In this study, similar LR and LPR would indicate a general agreement among the labs. Physical parameters were found to be the only category tested with a similar LPR among the labs. Physical parameter measurements indicate significant pressure drop variability for the tested cigar products which would naturally lead to high variability for smoking analytes. Tobacco testing variability was higher than that noted for physical parameters but in line with results from more extensive collaborative studies. LPR differences among the labs indicated product variability and/or differences in sensitivity of methodology for discerning the products. The resulting smoke yields with high variability, high LR, and varied LPR reflected the broad design differences, inherent complexity of cigar smoking, and lack of standardized, and in some cases robust, methodology.

Based on the results of this study and the relatively limited information in the literature, additional research related to the standardization of cigar methodology and the establishment of foundational data for reference products is warranted. Given the wide range of design parameters, the inherent variability of the product category, and the relatively small number of testing laboratories, it is anticipated that acceptable *r* and *R* values for smoke analyte methodology will likely be much broader and more challenging to establish compared to other tobacco-product categories.

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CONFLICT OF INTEREST

At the time of the study all authors were current employees of ITG Brands LLC.

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