Temporal variability of analytical testing for e-vapor products and impact on number of replicates

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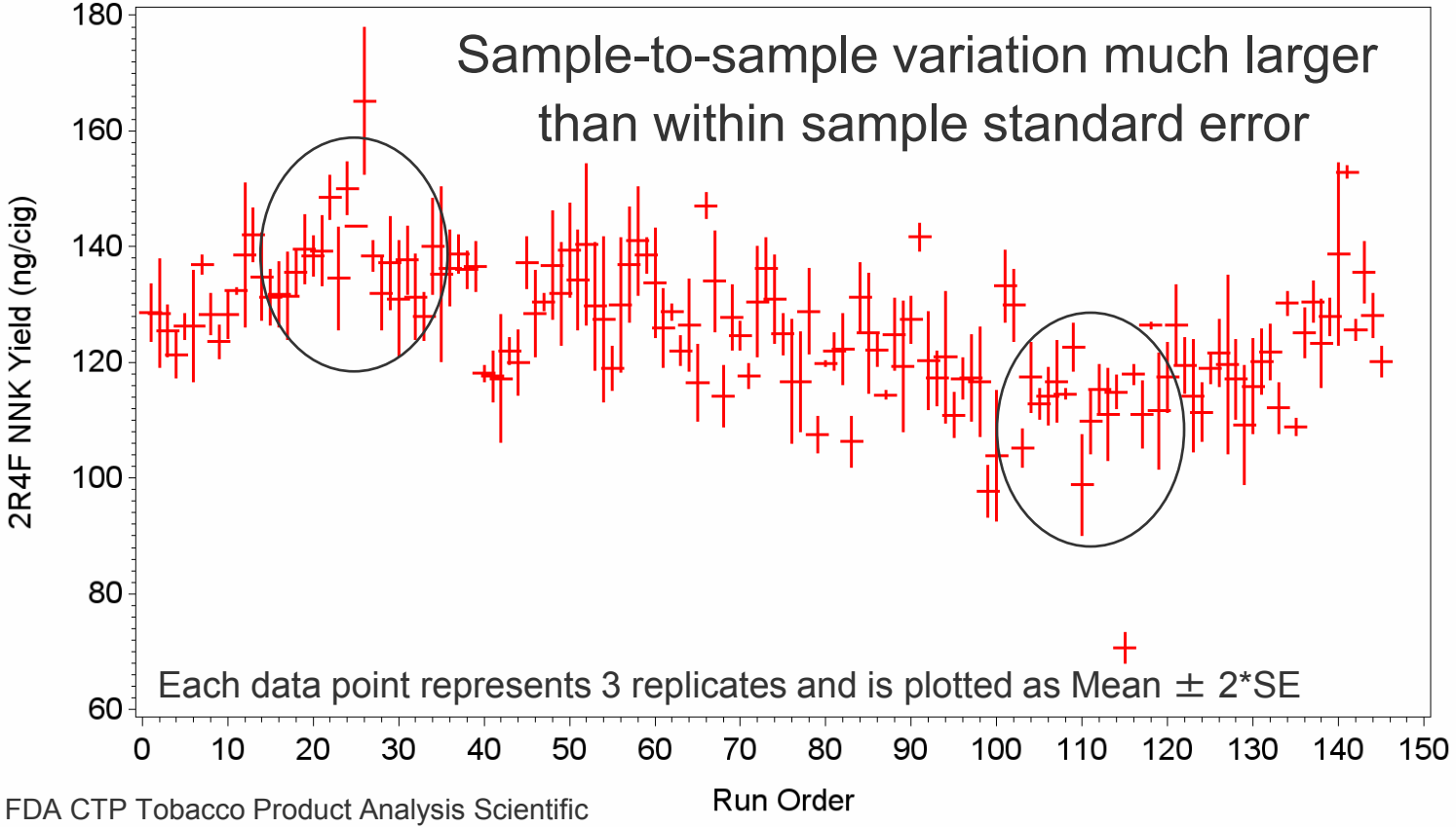
Testing for E-Vapor Products

- FDA/CTP PMTA ENDS draft guidance recommends that testing should be based on three different batches with a minimum of 10 replicates per batch.
- The reason for doing replicates is to improve the precision of the resulting estimated values.
- However temporal variability of analytical methods limits the effectiveness of additional replicates in improving precision and complicates the analysis comparing the product at different time points.

Laboratory Variability

- Variability using the same laboratory, same operator, same equipment, same materials over the shortest practical period of time is called *repeatability*
- Variability using different laboratories and implicitly different operators, different equipment, different materials is called *reproducibility*
- Anything in between with some of the factors potentially influencing the results changing but not all of them is called intermediate precision.
- A form of intermediate precision can be examined through the repeated analysis over time of a reference product, possibly used as a QC sample – call this variability “temporal variability.” Could also think of this as method instability.

Illustration of Temporal Variability



Shown in: FDA CTP Tobacco Product Analysis Scientific Public Workshop, April 12, 2012

Temporal Variability and Reproducibility

- Over a long span of time, temporal variability within a lab often approximates the lab-to-lab variation seen in collaborative studies

Temporal variability within lab

NNK smoke yield (ng/cig) of 2R4F		
Mean	r (% of mean)	R (% of mean)
125.7	13.2%	28.6%
Based on within Lab temporal variation		

Collaborative study results

NNK smoke yield (ng/cig) of 3R4F		
Mean	r (% of mean)	R (% of mean)
97.1	15.0%	31.4%
From CORESTA Recommended Method No. 75		

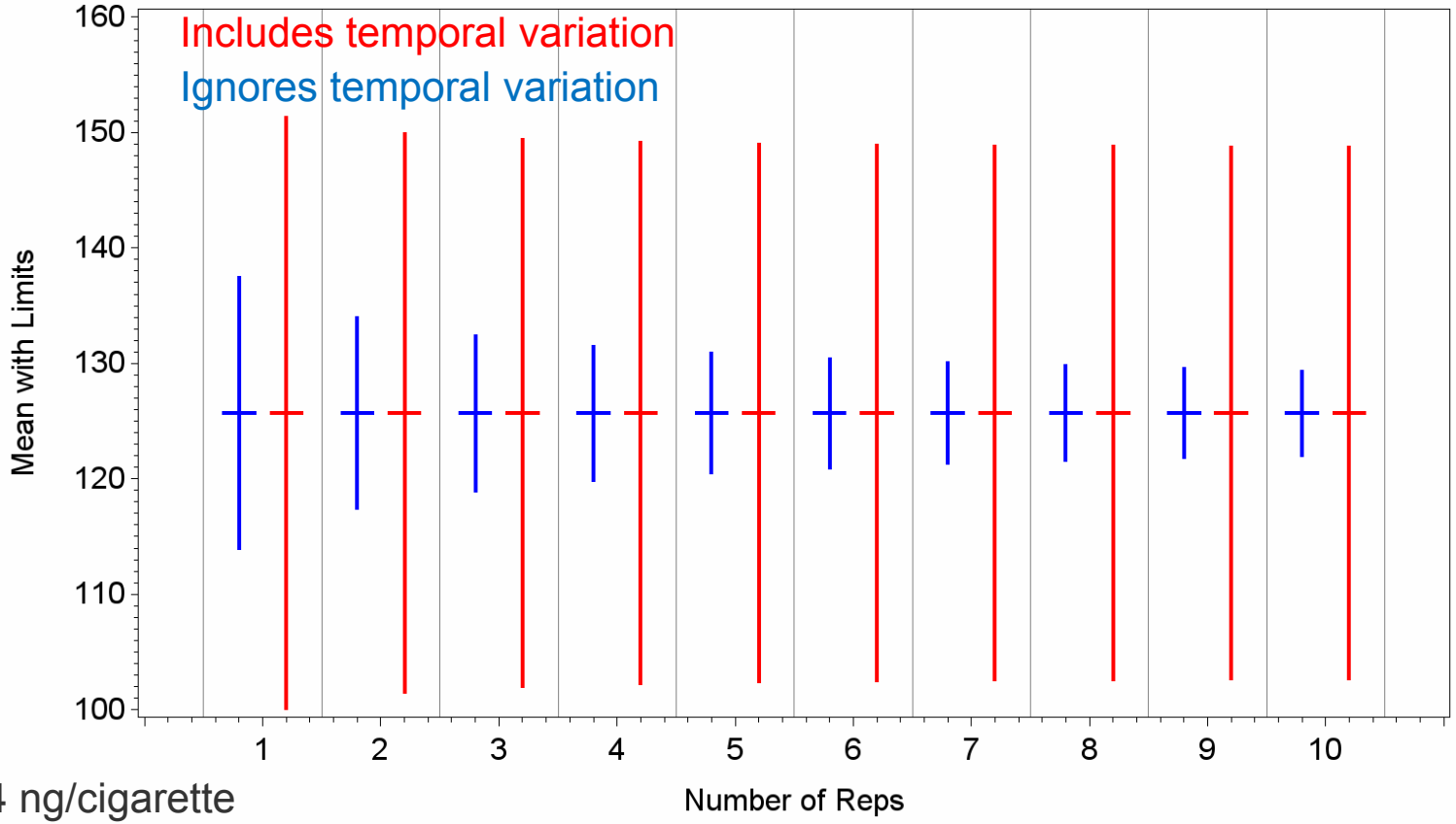
Effects of Temporal Variability on Uncertainty

- The (naïve) expectation for the uncertainty associated with replicate testing could be $\sigma_{\bar{y}} = \sqrt{\sigma_e^2/n} = \sigma_e/\sqrt{n}$
  - The uncertainty appears to get quite small with replicate testing – but only by ignoring temporal variation (method instability)
- When testing is carried out within a short period of time the uncertainty in the test result $\bar{y}$ (average value of the replicates) is given by

  $\sigma_{\bar{y}} = \sqrt{\sigma_T^2 + \sigma_e^2/n} > \sigma_T$

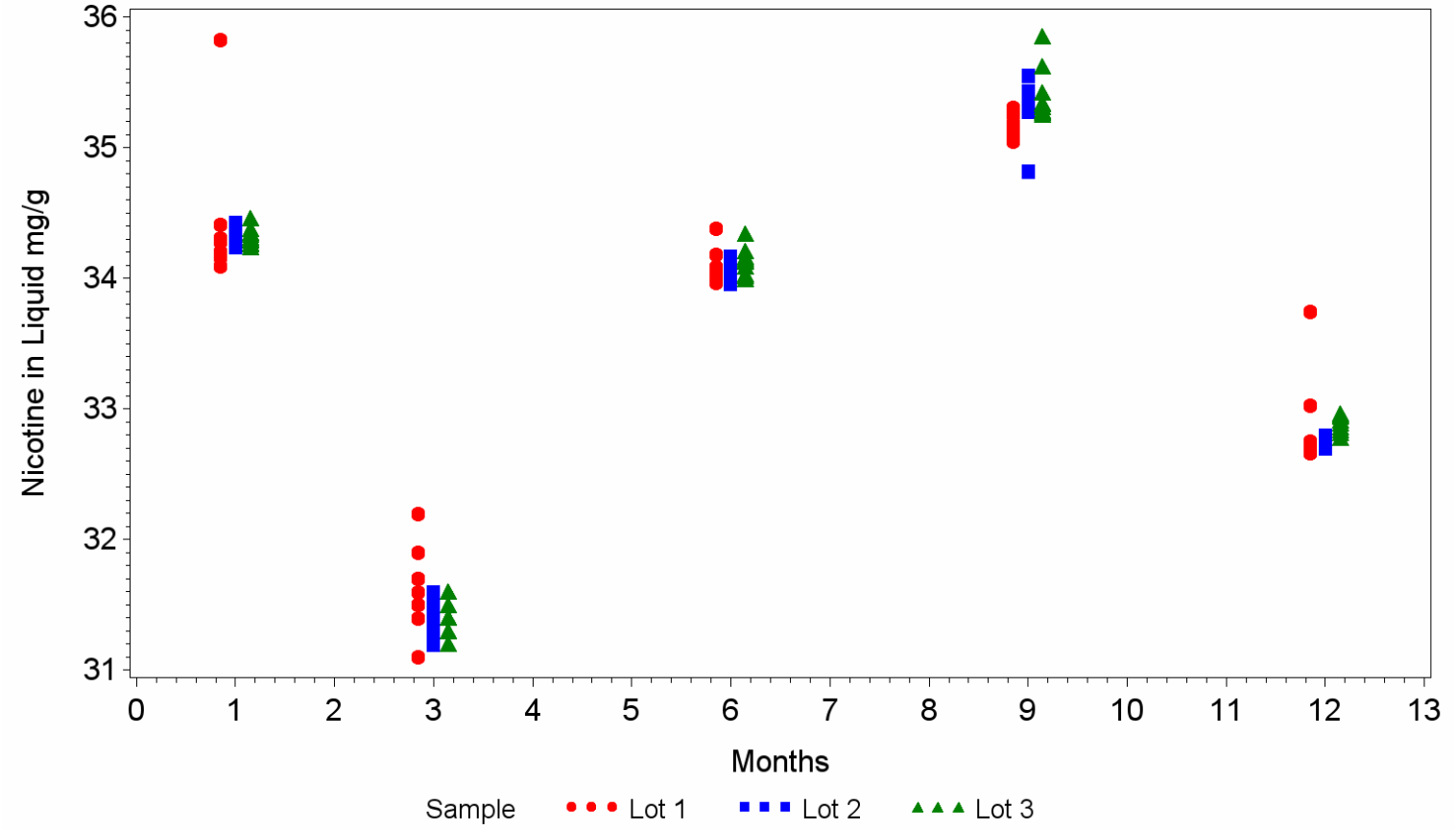
  where σ_T is the temporal variation term and σ_e is the short-term variation (analogous to the repeatability standard deviation)
- That is, when testing is carried out in a short time period, the resolution can be no better than the temporal variation, no matter how many replicates

Confidence intervals for mean with or without temporal variation



σ_T=11.4 ng/cigarette

Temporal Variation Giving Apparent Differences in E-vapor Liquid



What affects the utility of additional reps?

- The ratio of the temporal variation to rep-to-rep variation determines the utility of additional replicates
  - The larger the temporal variation is a proportion of the rep-to-rep variation, the less useful are additional replicates

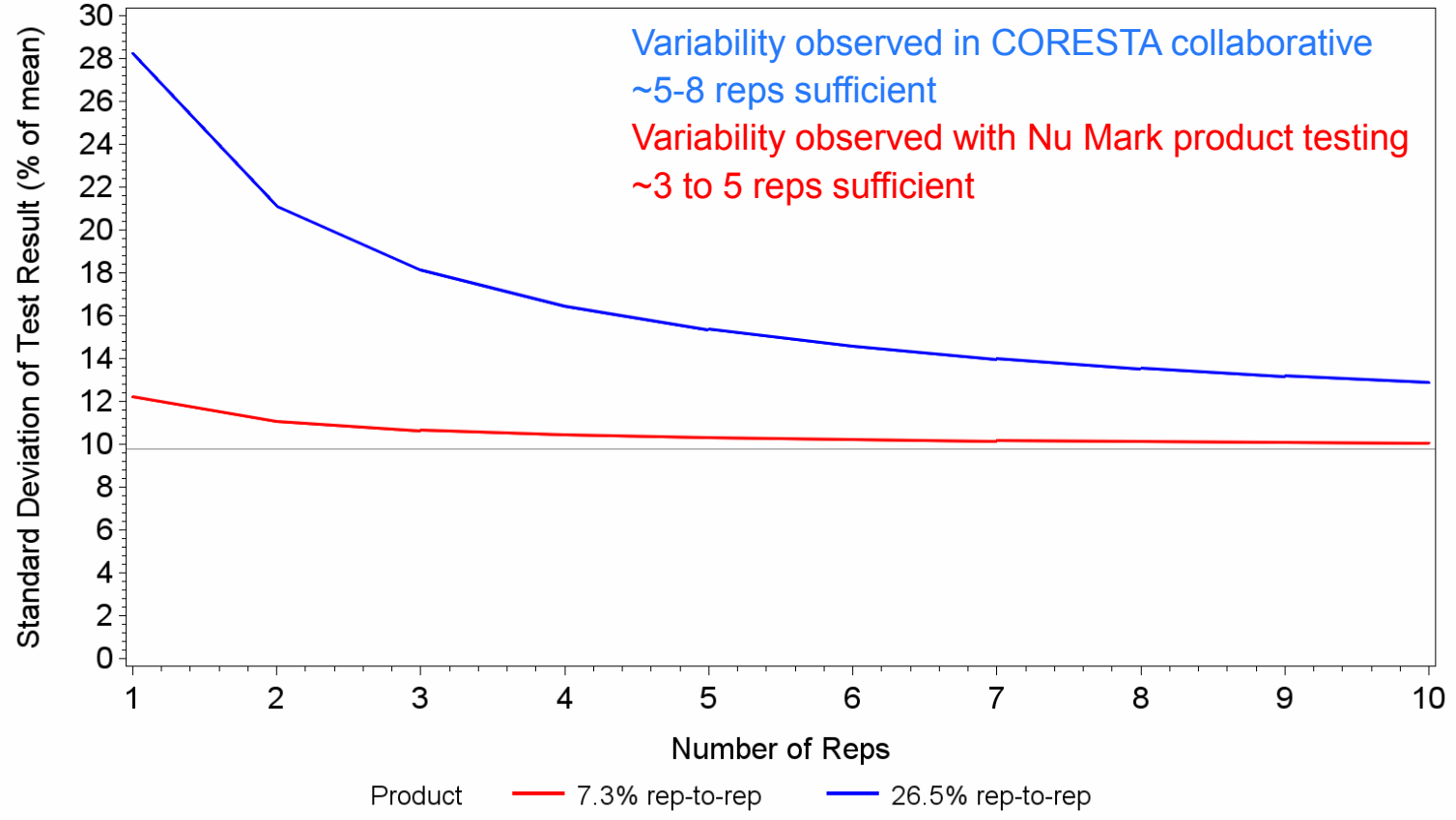
How to estimate temporal variability?

- When available, the variance components can often be estimated using long-term QC data in the lab
- Alternatively, the variance components coming from collaborative studies can approximate the temporal variation

E-vapor Products Nicotine in aerosol

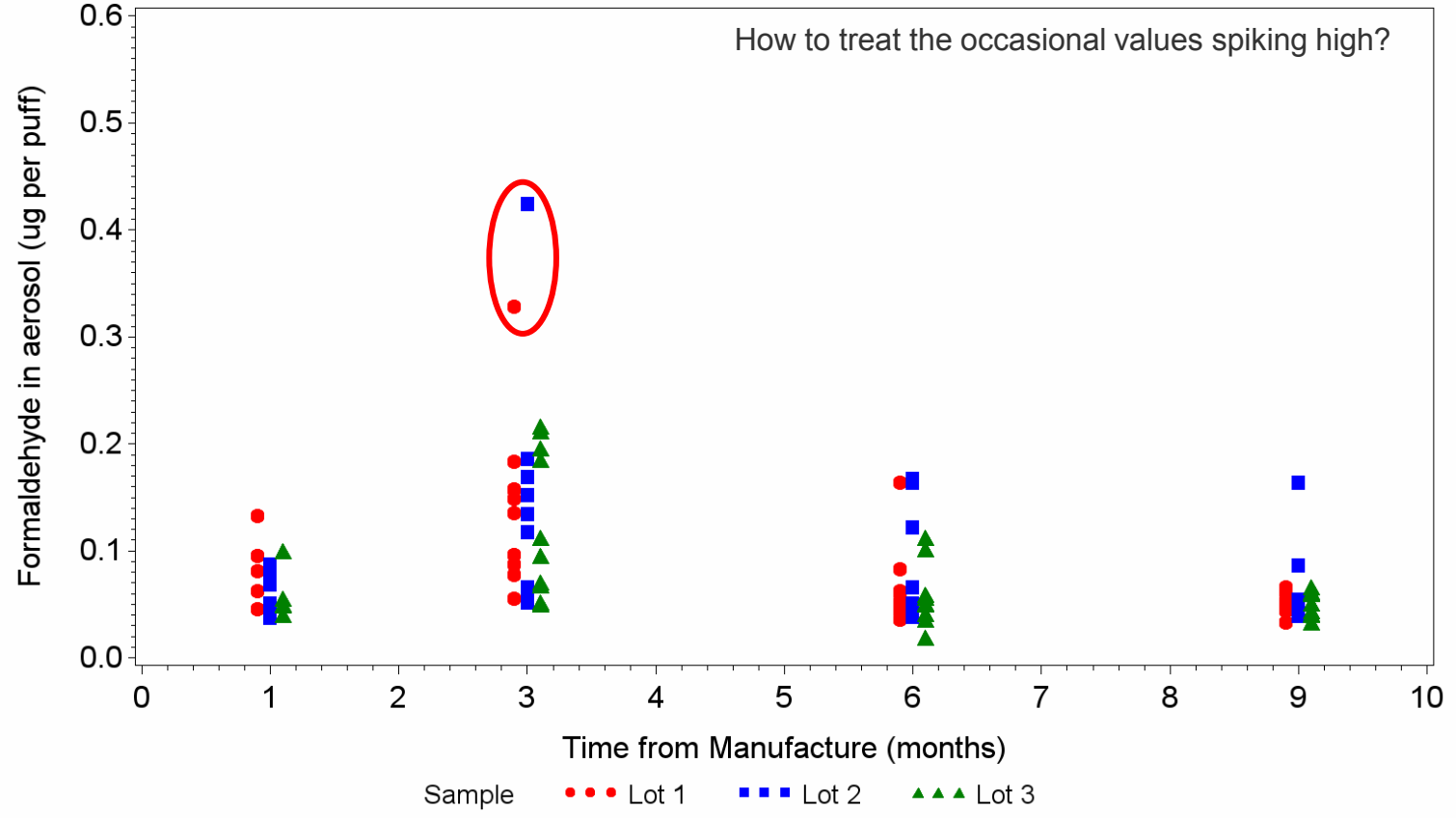
- CORESTA E-vapour Sub-Group 2015 collaborative study
  - Lab-to-lab variation of nicotine was 9.8% of the mean.
  - Rep-to-rep standard deviation of nicotine averaged 26.5% of the mean.
- Separate testing of Nu Mark products has shown rep-to-rep standard deviation of nicotine in the aerosol averaging 7.3% of the mean.

Estimated Effect of Additional Replicates for Nicotine in aerosol*



* Temporal variation estimated to be 9.8% of mean

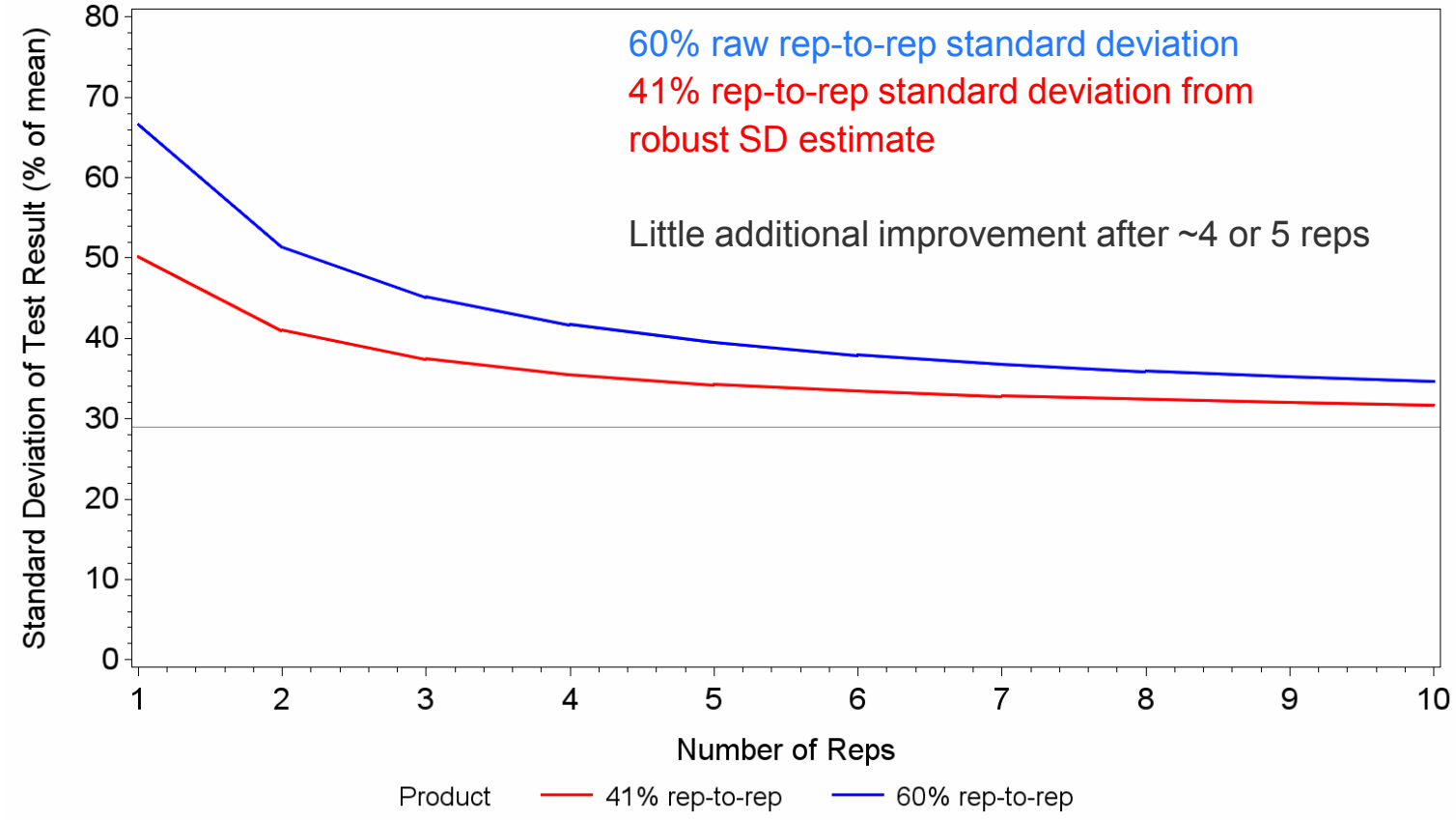
Formaldehyde results



E-vapor Products – Formaldehyde in Aerosol

- To date there have not been collaborative studies on carbonyls such as formaldehyde in e-vapor aerosol
- As a first approximation use collaborative study results in cigarette smoke.
  - Lab-to-lab standard deviation for formaldehyde is estimated to be 29% of the mean from the collaborative study referenced in CORESTA Recommended Method No. 74.
- Based on testing of Nu Mark products, replicate-to-replicate variation has been:
  - 60% of the mean based on the raw data values
  - 41% of the mean based on using robust estimators that down-weight the extreme values

Estimated Effect of Additional Replicates for formaldehyde in aerosol*

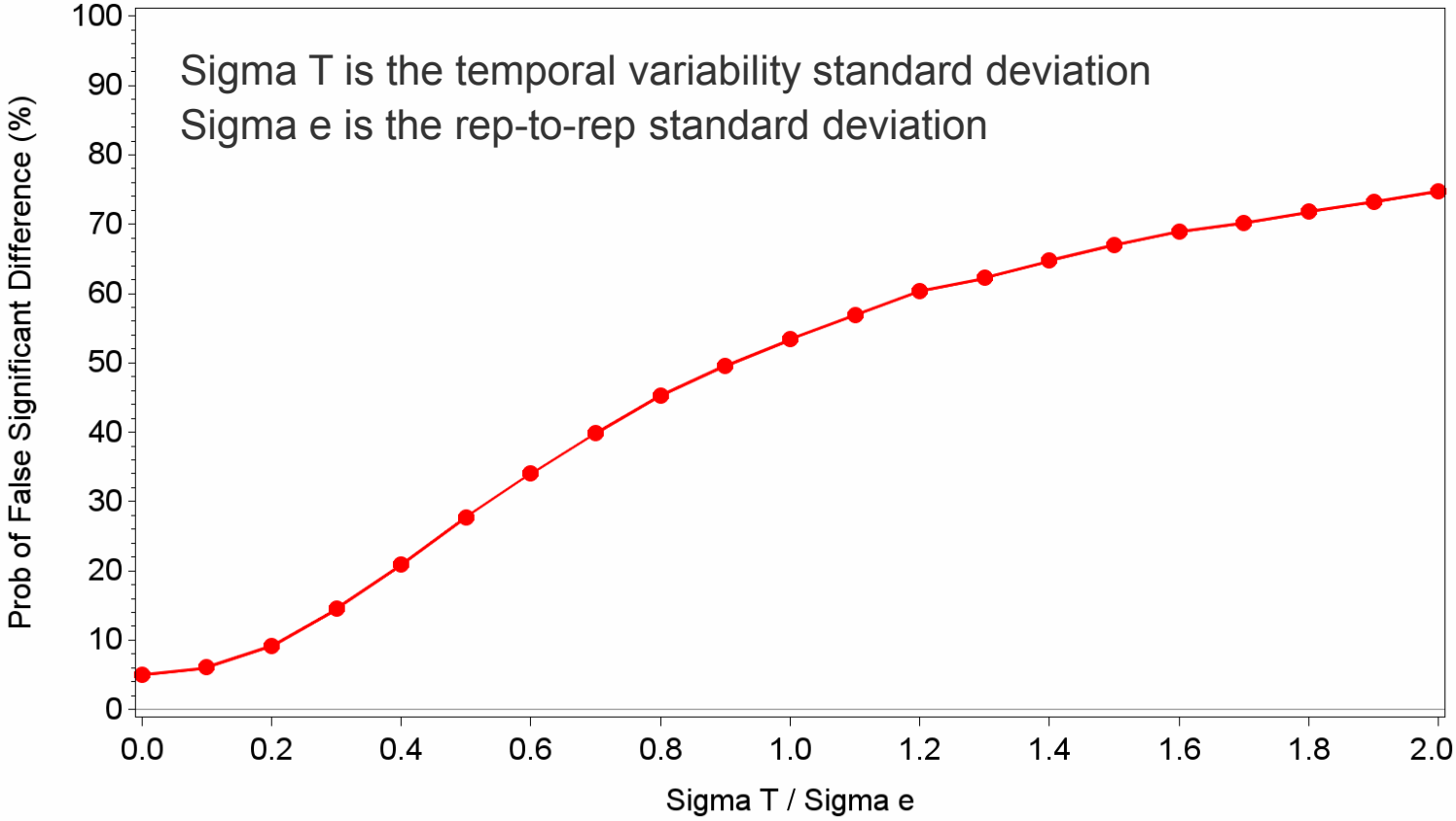


* Temporal standard deviation estimated to be 29% of mean

Analysis: I can just do a t-test, right?

- Many common statistical techniques (such as a two-sample t-test or one-way analysis of variance) make the implicit assumption that there is no temporal variability in analytical methods
- Temporal variability causes the standard statistical tests to give misleading results.
  - That is because the tests effectively use the "wrong" variability
  - Those tests use something akin to $\sigma_e/\sqrt{n}$ as the standard error when they should use something akin to $\sqrt{\sigma_T^2 + \sigma_e^2/n}$
- The effect of ignoring temporal variability will be greater, the larger the ratio of the temporal variability to the rep-to-rep variability: σ_T/σ_e

Probability of t-test finding a difference when there is none



Calculated by simulation, assuming 10 reps per group

Analytical Alternatives

- If stability samples can be stored in way that keeps them from changing, all time points can (theoretically) be analyzed at the same time and temporal variability avoided
  - I.e., stabilize samples at time 0, 3 months, 6 months, etc., then analyze them all of them at the end at the same time.
- If there is a stable reference product, the reference product analysis can serve to anchor the analytical method
  - Simple in theory, more difficult in practice.
  - Variability of reference product analysis must be taken into account.
- Temporal variability can be assessed and explicitly accounted for.
  - Likely through either lab QC data or collaborative study data
- Judge stability by consistency of pattern across products rather than product-by-product

Summary

- Temporal variability is inevitable with any analytical method.
- In the presence of temporal variability, comparing test results tested at different time points is difficult and requires the temporal variability to be taken into account
- When comparisons are made from testing at different time points, there are sharply diminishing improvements to the precision of the estimated values from additional replicates
  - In many instances, testing 3-5 replicates provides almost as much precision as testing 10 or more replicates.
- Temporal variability can cause standard statistical analyses to give misleading results by falsely attributing shifts in the analytical method to product differences.
- Options were suggested as potential alternatives to carry out the analysis of stability results accounting for (or avoiding) temporal variability in the analytical method.
- Standardized protocols should be developed for conducting and analyzing e-vapor stability and other studies requiring comparison of products at different time points.

References

- Michael Morton, “Variability Observed when Analyzing Reference Materials for Tobacco Specific Nitrosamines (TSNAs),” FDA CTP Tobacco Product Analysis Scientific Public Workshop, April 12, 2012.
- CORESTA E-Vapour Sub-Group Technical Report – 2015 Collaborative Study for Determination of Glycerin, Propylene Glycol, Water and Nicotine in Collected Aerosol of E-Cigarettes
- CORESTA Recommended Method No. 74 – Determination of Selected Carbonyls in Mainstream Cigarette Smoke by High Performance Liquid Chromatography (HPLC)
- Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems: Guidance for Industry. Draft Guidance May 2016. U.S. Department of Health and Human Services Food and Drug Administration Center for Tobacco Products.