THERAPEUTIC DRUG MANAGEMENT AND TOXICOLOGY DIVISION NEWS

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Therapeutics & Toxins News

Newsletter for the TDM and Toxicology Division of AACC

In This Issue:

Quality Control for Multi-analyte LC-MS/MS Methods

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Clinical laboratory methods to set quality control (QC) confidence bands for single analytes are well established[i,ii]. However, setting QC confidence bands when many analytes are quantified by a single method, as in LC-MS/MS metabolomic profiling[iii], steroid profiling[iv], and drug testing[v], is less well understood. QC confidence bands for multi-analyte methods exist on a continuum bounded by methods where QC results for all analytes are perfectly correlated, and methods where QC results for all analytes are perfectly independent. If QC results for many analytes are perfectly correlated, confidence bands reduce to the single analyte case. But if QC results for many analytes are perfectly independent, the rate of QC outliers rises, and acceptable QC results for all analytes become progressively less likely as the number of analytes increases. For example, the probability of n non-correlated analytes all meeting a 95% confidence band in a single QC level is 0.95^n. Expected outlier rates at the boundaries of perfect correlation and perfect independence for a method with 35 analytes are shown in the table. Multi-analyte QC results are seldom at the boundaries of perfect correlation or perfect independence; they display varying degrees of correlation across analytes. Outlier rates are highly dependent upon where the QC data's internal correlations are located on the continuum between perfect correlation and perfect independence. This communication describes several approaches to multi-analyte QC confidence bands as well as their strengths and limitations.

Confidence Bands and QCs for 35 analytes	Expected Fraction of Batches With One or More Outliers Assuming Perfect Correlation	Expected Fraction of Batches With One or More Outliers Assuming Perfect Independence
Conventional 95% confidence bands (+/- 2 SD for each analyte)		
One QC or two QCs with individual analyte results perfectly correlated between QCs	5.0%	83.4%
Two QCs, assuming no correlation of individual analyte results between QCs	9.8%	97.2%
Conventional 99.7% confidence bands (+/- 3 SD for each analyte)		
One QC or two QCs with individual analyte results perfectly correlated between QCs	0.3%	10.0%
Two QCs, assuming no correlation of individual analyte results between QCs	0.6%	19.0%
Bonferroni correction 95% method confidence band		
One QC or two QCs with individual analyte results perfectly correlated between QCs	0.0%	5.0%
Two QCs, assuming no correlation of individual analyte results between QCs	0.0%	9.8%

Preliminary assessment of a QC data set should include basic tests for non-normality (mean vs. median, kurtosis and skewness), a correlation matrix of QC results across analytes for the data set, and an evaluation of the degree of individual analyte result correlation between multiple QCs, if used.

Some laboratories employing multi-analyte methods initially establish QC confidence bands for each analyte (e.g. 95%, as shown in the table), and are met with frustration when most batches contain QC outliers. The QC outlier rate is related to the number of analytes and the degree of QC analyte results non-correlation. Use of two QC levels, as recommended by CLSI, can increase the outlier rate. In our experience, r^2 between results for a single analyte at two QC levels at different concentrations, analyzed many times, is usually statistically significant but << 1.00. Thus, the impact of using two QCs vs. one QC on the outlier rate is noticeable, but not as marked as if $r^2 = 0$.

The next step may be to expand the individual analyte confidence bands (e.g. 99.7%, as shown in the table), which will reduce the number of QC outliers. However, depending on the multi-analyte method in use, this may or may not result in a practically useful QC outlier rate.

One method to account for the effects of multiple analytes on overall method confidence bands is the Bonferroni correction for multiple comparisons, which is widely used in biostatistics[vi]. For an overall method 1-α confidence band (e.g. 1-α = 0.95), individual confidence bands for each of the n analytes are set at 1-α/n. For example, if five non-correlated analytes are measured in a single method, a 95% method confidence band, using the Bonferroni correction, would employ 99% confidence bands for each analyte. However, the Bonferroni correction effectively assumes zero correlation between analyte QC results and can be too forgiving when the analytes have non-negligible correlations, and may fail to identify true outliers.

An excellent practical alternative approach is to set a global percentage confidence band for all analytes (e.g. +/-30% in reference 5). But this does not allow for differential variance in QC results across analytes. Some analytes may have practically useful batch failure rates, while others never fail.

Applied mathematicians continue to study the problems associated with multiple confidence bands. Sup-t band confidence bands, recently developed in econometrics[vii], address differential variance across analytes. We have tested Sup-t bands using multi-analyte LC/MS/MS QC data sets, and the preliminary results are promising.

This communication outlines several approaches to multi-analyte QC. Failure to appreciate the statistical subtleties involved in multi-analyte method QC can be problematic. Further investigations are needed. Ideally, these should employ actual production multi-analyte QC data sets.

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5. Enders JR, Smith JP, Feng S, Strickland EC, McIntire GL. Analytical Considerations When Developing an LC-MS/MS Method for More than 30 Analytes. JALM 2018 Jan; 2(4): 543-554.

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[vi] Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995; 310:170.

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Editor's Corner:

Dear Readers,

The Division Events were very successful and well-attended. The Division Table at the AACC Opening Mixer & Division Networking Event had many fun activities. The Division Annual Meeting and Luncheon had good talk and food for the members. And Division ePoster session, a new event replacing Poster Walk was full.

Division **Elections** open on Nov 15, 2019 and close November 22-December 6. Ballot link will be sent via Artery post or email to division members. Don't forget to vote!

-Pradip Datta, Editor.

Late-breaking election news:

The following nominations have been received so far:

For Secretary:

(Reelect) He Sarina Yang, PhD, DABCC. Assistant Professor of Pathology and Laboratory Medicine. Assistant Director, Central Lab and Clinical Chemistry Services. Director, Toxicology and Therapeutic Drug Monitoring. Weill Cornell Medicine

For Division member-at-large:

1. Gerard Meenan (see Biography below)
2. Alejandro Molinelli
3. Kimberly Robiyak

Bio: Gerard Meenan works as a laboratory manager at Pinnacle Testing, Inc. in Delray Beach, FL. He joined AACC in 2009. He is a member of the Society of Forensic Toxicologists. He has worked in the TDM and Toxicology fields for over 40 years. His experience includes work at major medical centers, independent reference laboratories, racehorse drug testing for Cornell University and as an expert witness in Toxicology testing. He has numerous publications and presentations about his research in TDM and Toxicology methods. His interests include the testing of opioids and novel psychoactive substances by LC-MS/MS.

AACC TDM TOX Web Resources:

https://www.aacc.org/community/divisions/tdm-and-toxicology/

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The editorial board invites ideas and article contributions for this newsletter. Please contact Dr. Pradip Datta at pradip.datta@siemens.com.