„Introduction to Standardized Data Analysis in Targeted Metabolomics“

Biocrates webinar on September the 6th, 2016 - Questions and Answers

ANALYTICAL THRESHOLDS

How is the limit of detection (LOD) defined in the Absolute IDQ® p180 Kit? How can I get the LOD information as service customer with no access to the MetIDQ software?

Different methods for the estimation of the LOD are available. We use 3 times the median of the concentrations in the zero sample for each metabolite. As a Kit customer, LOD values are readily available within MetIDQ software. As a customer of Contract Research, LOD values can be obtained from our Contract Research team upon request.

Is there any difference in using random values for imputation of values below LOD or simply using LOD/2?

Yes there is a difference. Fixed values do not properly represent the variance within the data set and may reduce the statistical power of the test. Random values on the other hand maintain variance within the data, while allowing unbiased tests.

How do you define the variability for a group when imputing values <LOD?

We either use normally distributed values or impute via a log spline function.

BIASED MEASUREMENT RESULTS

Why and when do I need batch correction?

When measuring several plates, slight deviations may occur particularly in the FIA part, since metabolite concentrations depend on sample preparation (for example in pipetting), machine settings and particularly lab and instrument conditions, as well as on other factors. Therefore an interplate batch correction might be necessary and can be achieved through QC normalization.

Is it possible to use samples from non-fasting individuals? Can samples be corrected for non-fasting?

Ideal is the use of samples from fasting individuals (>6hrs), taken at the same time of the day (circadian rhythm), as samples obtained postprandially may show shifts in concentrations and increased variance. In reality we frequently measure samples from longer archived samples with unclear collection history. We apply quality scores to identify outlier samples as well as overstored samples.

Isn't it possible to detect preanalytical outliers with multivariate outlier tools such as PCA?

In some cases it is indeed possible to identify preanalytical outliers using PCA. However, with the preanalytical quality markers discussed in the webinar, outliers would be detected that might be missed with the PCA. For example when the whole sample pool is affected a PCA will miss those samples and it will later on result in problems if the results are compared to another study, where no preanalytical issues occurred.

How does serum compare to plasma and blood for stability within the AbsoluteIDQ® p180 Kit?

Though our preliminary in-house study on preanalytical quality markers did not include serum as a matrix, we would still like to share the knowledge we gained from other studies. Plasma sample collection can be easily standardized, while the clotting process makes standardization of serum sample collection more difficult, e.g. based on differences in time until clotting and differences occurring during the clotting process. On the other hand, previous studies demonstrated that serum is slightly less susceptible to freeze/thaw cycles compared to plasma samples.

Generally, removal of blood cells by centrifugation of blood and the accompanying removal of haemoglobin and glutathione oxidase system makes plasma and serum more susceptible to chemical oxidative processes than blood. Hence, handling time after centrifugation should be kept to a minimum.

Also, please refer to Breier et al who performed a study on preanalytical effects on human serum and plasma samples using the AbsoluteIDQ® p180 Kit (Breier et al, 2014, Targeted metabolomics identifies reliable and stable metabolites in human serum and plasma samples, PLOS ONE).

Biocrates KITS AND SERVICE

Are there Kits available for other manufacturers of MS systems, such as Bruker or Thermo?

The Biocrates targeted metabolomics kits are currently available for Waters, AB Sciex and Thermo triple quadrupole instruments. We are working on to expand the portfolio for validated instrument platforms. You will be informed about new launches for additional instruments on our website or newsletter.

How many of my samples can I measure on a p180 Kit plate?

Quality controls, Blanks and Standards take up some of the wells on a p180 Kit plate. Depending on the number of replicates of quality controls you measure, 80 samples can be measured on a kit plate. We also offer half Kit plates in case of low sample number, which allow measurement of 40 samples. According to guidelines for bioanalytical method validation (e.g. EMA or FDA), we recommend loading a quality control after every 20 samples.

For what kind of disease indications the p180 Kit is most suitable? Or in other words, why the p180 kit metabolites show alterations in so many diseases?

The AbsoluteIDQ® p180 kit includes a combination of ~180 metabolites and metabolite classes addressing the central pathways, key rate-limiting-enzymes and common relevant pathophysiological processes (e.g. mitochondrial dysfunction, oxidative stress, insulin resistance, cancer cell signalling). Therefore, the AbsoluteIDQ® p180 kit is an excellent targeted metabolomics tool analysing metabolite alterations in broad variations of different diseases and indications as metabolic disorders (e.g. Diabetes, metabolic syndrome), cardiovascular diseases, neurology (e.g. Alzheimer´s disease) and oncology. As presented in the webinar the appropriate combination of metabolites to robust metabolic signatures enables the improved sensitivity and specificity of the metabolic read-out.

Do you have other data on accuracy of the analytics than on the amino acid data you have shown before?

The performance of the AbsoluteIDQ® p180 kit (acylcarnitines, glycerophospho- and sphingolipids, hexose, amino acids, biogenic amines) and Biocrates Bile acid kit was extensively tested in two international ring trials (p180 kit: Siskos et al. 2016, submitted; bile acids kit: Pham et al. 2016, J Appl Lab Med, in press) with a resulting inter-lab precision of 7.4% and 8.3% respectively. The AbsoluteIDQ® Stero 17 kit is tested on quarterly basis within a proficiency test program (Koal et al. J Steroid Biochem & Molecul Biology 2012).

Are there any distributors in the US from which we can purchase the kits?

We do have a sales organization in North America. Please contact sales@biocrates.com, and your request will be forwarded to the responsible person.

What other metabolites or metabolite classes can you measure?

Via our Metabolic Phenotyping Services, you can obtain quantitative data on more than 600 metabolites. Our analytical portfolio includes assays that cover the quantification of fatty acids, neurotransmitters, an extended

set of lipids, oxysterols, metabolites of the energy metabolism and vitamins, with the latter being for bioprocessing only. You will find a more detailed overview on our website or feel free to contact us for more information.

Is there any progress at adding more metabolites related to energy metabolism, such as citrate, lactate or pyruvate?

These metabolites are in the panel of the new energy metabolism in-house assay, where customers as contract research service have access to.

DATA EVALUATION

Do you have a standard set of sums and ratios that you apply in your data analysis?

Yes, we do have a large set of biologically meaningful sums and ratios, including ratios for assessing sample quality that we routinely check when we analyse a dataset. As a contract research customer there is the option to include these checks and further interpretation in your data analysis package. For details please contact our sales team at sales@biocrates.com.

In what aspects is MetIDQ software different from XCMS?

The Bioconductor XCMS package is basically a framework for processing chromatographically separated mass spectral data. You will need to assemble or customize your pipeline programmatically without a graphical display. Furthermore, it is intended for untargeted profiling. We on the other hand provide software with a graphical user interface which assists you step by step in the kit workflow, thereby allowing you to perform targeted metabolomics analysis with calibration standards. MetIDQ contains all data about standards and controls in the kit and thus can conduct automated standard curve creation and quality control. In addition, it features result visualization and normalization, building metabolite signatures or ratios and data export to different formats, as well as a basic statistical analysis.

Please follow the link below for detailed information on MetIDQ:

http://www.biocrates.com/products/software