BP4NTA
Workshop Report

Non-Targeted Analysis: Building bridges among mass spectrometrists

Wednesday, June 5th, 5:45-7:00pm

Coordinators: **Dr. Ruth Marfil-Vega**, Past-Chair, BP4NTA; Sr Market Manager – Environmental, Shimadzu
Dr. Christine Fisher (O'Donnell), Past-Chair, BP4NTA; Chemist, Food and Drug Administration
Dr. Gabrielle Black, Operations Liaison, BP4NTA; Chemist, United States Geological Survey
Presenters: **Dr. Christine Fisher (O'Donnell)**, FDA
Dr. Jonathan Martin, Stockholm University
Dr. Nina Zhao, University of San Diego

Initial/Final Attendance: 100-120.

Audience Composition:

- Academia, industry, government; organizations represented (non-exhaustive list): EPA, FDA, USGS, NIEHS, Agilent, Shimadzu, Thermo, TofWerk, multiple US and international universities, Metabolon, members of the Periodic Table of Food Initiative…
- Most attendees were established and young professionals in their fields.

Figure 1. Example stickers

As attendees entered the workshop, they were asked to choose their favorite "flavor" of quality control/assurance parameters for Non-Targeted Analysis (NTA) and given the opportunity to make a wearable sticker (Figure 1) reflecting their choices. Each "flavor" option was printed on sticker in the shape of a scoop of ice cream, and wearable stickers allowed users to add up to three ice cream scoops on top of an ice cream cone. Attendees were also asked to record their top three choices in an online poll (platform used: Slido, free account with maximum number of responses equal to 100). The options provided intentionally represented large categories, and participants were asked to think about which category no NTA project could do without. The options were: Blanks, Non-Extracted Internal Standards, Extracted Internal Standards, Pooled Samples, Analytical Replicates, Standard Chemical Mixes, and Other. The opening activity (build your ice-cream) allowed for attendees to start talking about the topic of the workshop before the general discussion started. As some attendees continued wearing the stickers after the workshop, it catalyzed further conversations at the conference. Additionally, results from the poll were used to drive the general discussion.

Coordinator Ruth opened the workshop with brief welcoming remarks and introduced the aim of the workshop - to discuss the various quality control and quality assurance measures implemented in the field and the future for potential standardization or harmonization among researchers. She provided a

brief introduction to the group Best Practices 4 Non-Targeted Analysis (BP4NTA) and the overlap of the group's goals with those of the Exposomics Interest Group, as well as other fields using NTA.

Drs. Christine Fisher (O'Donnell), Jonathan Martin and Nina Zhao were then introduced, and each shared a brief (<5 minute) perspective of how they implemented QA/QC in their research and posed questions to the audience to promote discussion.

Christine shared hers and BP4NTA's perspective (food analysis and multidisciplinary approach, respectively) of QA/QC practices in NTA, stating that no current standardization of practices exists, and it is somewhat a "wild, wild west" when it comes to what researchers are implementing. Christine provided an update on the ongoing effort to create a standardized chemical mix and asked the audience their opinions on having a standard mix to be used across all labs, researchers, fields, and projects.

Jonathan provided his perspective as an exposomics and environmental analysis practitioner, sharing that in each step of a traditional nontargeted analysis workflow, QA/QC guidelines exist from other fields that can be leveraged. He also shared his insightful opinion that the field is relatively young and implementing a standard 'one size fits all' QA/QC approach at this point would be a disservice because we should allow researchers to innovate and explore the best ways to assure quality in datasets.

Nina, currently working in metabolomics and "big data" analysis, presented an example of how manual 'spot checks' have caught errors in data post-processing of complex computational models, databases, and processes and that it is unlikely that anything will replace the benefit of expert knowledge and manual evaluation of results.

Following the three short presentations, the results of the online poll were displayed (Figure 2), and a discussion was started on why participants chose what they did and how different facets of each option played into ensuring quality data.

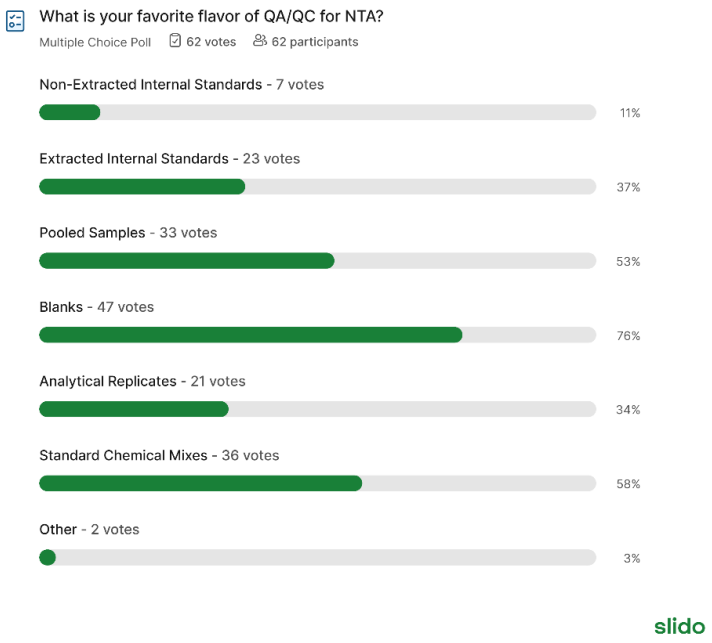

Figure 2. Summary of poll responses.

The first topic discussed was Blanks since it was chosen the most frequently. Main topics of discussion were types (e.g. field, method, injection) and frequencies, how each type is used, the importance of blanks representing every step of the sample preparation, data acquisition, and processing steps, and confidence in blank subtraction versus blank filtering. The audience agreed that “blanks” is a broad topic and there is not a common approach among disciplines for using them as a QA/QC parameter. Many participants (indicated by a show of hands) indicated that they do not trust blank subtraction. The conversation was very detailed and we could have filled the entire allocated workshop time with this topic alone. This highlights the need for harmonization of a naming system for “blanks” and development of recommendations from multiple fields doing NTA.

Standard Chemical Mixes were discussed next and focused on how chemical standards are currently used to evaluate method suitability and chemical space. It was debated whether a single standard mix could be used by all researchers. Ultimately, participants were divided on whether a single mix would be appropriate for all projects. There seemed to be some agreement that a standard mixture may need to be tailored to the method type due to variability in detectable chemical space; however, a single standard mixture may be able to bridge different fields (e.g., exposomics, food, metabolomics, environtmental analysis, etc.) using the same method type. In post-workshop discussions, some attendees mentioned that although they would love to have a commercially available standard mixture to prevent them from having to make their own mixture every time, a single “conflicting” compound could prevent them from using the mix (e.g., a standard that is incurred in their sample matrix and/or is too close in mass to an incurred chemical). It was also suggested that it would be beneficial to include compounds that can test differences between instruments (e.g., differences in heat/energy of instrument sources which may lead to differential in-source fragmentation). There is clearly interest in this topic and a lot of different needs/uses to consider for a standard mixture to be more broadly applicable, so there is a need for continued discussion and feedback.

The use of Pooled Samples was discussed and audience members shared the various uses of these samples - evaluate sample response variability, matrix effects, batch normalization methods, and as a comparative sample to highlight unique features in individual samples. This QA/QC is more common in specific fields and some attendees shared how, as their area of work has changed over time, they learned the benefits and starting using pooled samples. Similar to the blanks discussion, the engagement of participants from different fields enabled cross-pollination of ideas.

Participants that chose “Other” (3%) shared that some of their QA/QC practices also include Standard Reference Materials (SRM), acquiring large quantities of the same sample that can be reextracted across projects and days to evaluate consistency of methods, and intensive evaluation and tracking of instrument performance.

There was overall agreement that ~40-50% of the samples analyzed in a batch are QA/QC samples. Representatives from for-profit organizations working on metabolomics stated they have this typical load of non-billable samples and that’s the reason for the high cost of this type of analysis. Cost/benefit analysis of some practices were not discussed as this is normally not a priority for R&D organizations; however, it will require additional discussion as the field grows and for-profit or standardization organizations get more involved.

Audience participation was excellent, with roughly 25 *different* individuals contributing to the discussion, and many stated the conversation could have continued for several more hours. Common themes that

continually resurfaced during the discussion were that expert knowledge and manual investigation is one of our best QA/QC tools available and that compound discovery requires a lot of QA/QC, and the struggle to choose only three categories in the poll was shared by all. Continuing to report practices implemented and how those practices promoted good data quality is crucial in developing shared methodologies in an expanding field. Forums, like this workshop, in which the NTA community from different fields (food, environmental, exposomics, metabolomics…) can convene to exchange information are essential for supporting the growth of NTA.

Notes from Exposomics Interest Group:

Coordinators: Ruth Marfil-Vega, Pablo Gago Ferrero

Attendance in this workshop was almost double compared to last year's.

As shared during the coordinators' meeting, three out of four mics didn't work and there was no AV help available. We used the laptop in the room with minor issues (the laptop would not maintain a duplicated display and switched automatically to "extended" when we switched windows (i.e., between the ppt file and the slido poll); we had previously collected the presentations from the speakers to minimize problems and maximize the time for discussion. We used Slido as the platform for interaction with participants.

The IG has started conversations with potential candidates to become the new co-coordinator with Dr. Pablo Gago-Ferrero. Once a new co-coordinator is confirmed, information will be shared with ASMS, at latest by the call Jennifer will schedule in September.