Validation Guidance App J (AOAC)

In App J, those laboratories opting to validate qPCR should be using sections 6.1-6.3.

Additionally, please see below for more information for validations that would meet AOAC guidelines:

1. Must use live cultures and each candidate method validated would need to also have a cultural confirmation performed as well.
   a. Cultural Confirmation should have: >5-day incubation on a non-selective fungal agar (PDA, SABDEX, etc.).
2. If there are multiple matrices, use a different organism for each matrix. **A mixture of both yeast and mold is required**.
3. Inoculum conditions and stabilization/equilibration:
   a. For flower matrices, the inoculum should be a dried culture (if not naturally contaminated) and the matrix should be equilibrated for 2 weeks after inoculation.
   b. Edible type products like gummies and chocolate should be inoculated with heat stressed cultures and also have a 2-week stabilization.
4. Number of replicates (5 reps at a minimum of 3 levels (naturally contaminated) or 4 levels (artificially contaminated). There should be a minimum of a 2 Log range for contamination. (e.g. average of 100 CFU for the low, 10,000 or higher CFU for the high level).
5. For qPCR methods specifically: must show that the standard curve is robust enough with a <u>wide range of strains</u>.
   a. There is a requirement for a different curve for each strain tested, this would require the matrix testing to be done with any claimed strains.
   b. This is to say the lab may not build curves for inclusivity and exclusivity with each strain, but only validate one in the matrix study.
6. Acceptance Criteria: 90% CI of bias between two methods must fall within -0.5 to 0.5 log10 for a given matrix at a given concentration.
   a. A minimum of 5 replicates is required per contamination level when determining bias and 95% CI
7. Inclusivity/exclusivity testing:
   a. A wide range of organisms in the countable range (inclusivity) or TNTC (exclusivity) of the method.
   b. This should be done by the method developer with a complete validation, but in this case, it will need to be completed by the laboratories.