STWG Objective #11 – Variables

1. Number of strains
   For qualitative studies with pathogens, it depends on the organism. It has been AOAC practice to require "inclusivity" testing of at least 50 target strains in pure culture and "exclusivity" of at least 30 non-target strains in pure culture. The collection of target strains should be representative of the breadth of the target group in terms of genetic or serological types. Non-target strains should represent those most closely related to the target group biochemically, serologically, or genetically, e.g., other members of the Enterobacteriaceae for the case of *Salmonella* spp. as a target. Target strains are to be cultured under the enrichment conditions specified for the test (selective enrichment if this is part of the procedure). Non-target strains are cultured under non-selective conditions to present a worst-case scenario.

   For inoculation of food samples, a different target strain is used for each food type. In the early years, there was an attempt to pair foods and strains based on historical illness outbreak or product recall data (e.g., *Salmonella* Enteritidis in eggs).

   These issues will be addressed by the BPMM Task Force as proposed guidelines are drafted.

2. Number of foods
   Historically, for *Salmonella*, normally 20 foods tested in a pre-collaborative study (or AOAC PTM study) plus 6 foods in a collaborative are viewed as sufficient to support claims for "all foods". Some within the AOAC review community are becoming uncomfortable with this, since it has been found subsequently that some methods approved for all foods have been shown to be ineffective for certain foods not tested in their validation studies. There has been talk of limiting approval claims to those foods actually tested. Restricted approvals are less helpful to the end user of the test, but obviously more accurately indicative of what is known about the test capabilities. It might be better to warn users that the procedure has been tested only in certain circumstances and users should be required to advise AOAC of genuine false negative or false positive findings obtained in subsequent investigations; this should then lead to the issue of an administrative warning concerning use of the procedure in such circumstances.

   The approach to *Listeria* has been a bit different. Normally 15 foods are tested, and claims are issued for product groups – meats and poultry, seafoods, dairy products, fruits and vegetables, environmental samples, etc.

   For *E. coli* O157:H7, the list of foods of interest is much narrower, generally limited to raw beef and perhaps sprouts and freshly pressed apple juice.

The BPMM task force does not prescribe a specific number of foods, but tends toward allowing the method developer to make a claim based only on those foods successfully validated. The Matrix Extension group has developed new food categorization schemes with associated rules for matrix extension (see Appendix B).

3. Inoculation levels
   The current AOAC requirement is for at least one level with 20 replicates where the results produce "fractional positives", i.e., less than 20 are positive by at least one of the methods (test or reference). This is taken as evidence that the majority of test samples contain not more than 1 cfu per 25 grams. In order to produce this result, analyst inoculates at ~ 1 cfu per 25 grams of product, sometimes higher if inoculum die-off is expected. The actual inoculation levels are estimated by an MPN determination. There is also a requirement for at least 5 uninoculated control samples. Normally, analysts prepare 2 levels of inoculation in the hope that at least one of the levels will produce fractional positives. The BPMM Statistics working group recommends that 4 levels of inoculation be used in estimating the LOD50: two levels with fractional recovery, one level all or nearly all positive and one level all or nearly all negative.

4. Choice of reference method
   Currently, in AOAC *Official Methods*SM pathogen test studies, the FDA BAM (*Bacteriological Analytical Manual*, available on-line), USDA MLG (Microbiological Laboratory Guidebook, available on-line) or appropriate AOAC *Official Method of Analysis*SM method is used as the reference procedure. In AOAC Research Institute *Performance Tested Methods*SM validations, other recognized official methods can be used, such as ISO or Health Canada. All of these are standard microbiological culture methods. Depending on the choice of reference method, the alternative method could compare differently. While the BPMM task force has not recommended any change in the choice of reference method, it does offer validation methods appropriate for cases in which no reference method is available. When a reference method is available, it will be included in the validation study and its performance will be evaluated alongside the alternative method.

5. Manufacturer of culture media
   Typically, the culture media used in a validation study is stated in the validation study report. However, the media source is generally not specified in terms of the method approval, i.e., there is a general doctrine of equivalence. This is a tough problem, because we know that there are differences in media performance from manufacturer-to-manufacturer and also lot-to-lot, which cannot be controlled. It is appropriate to note in the published AOAC method that medium from Manufacturer X was used in the validation procedure (which cover several batches of that medium) and that any laboratory which intends to use the procedure with media from other manufacturers must first check the alternative medium against that from Manufacturer X.

6. Manufacturer of reagents
   AOAC Official Methods of Analysis are supposed to be described in a generic way. So, in theory, someone could copy the method described for a commercial kit and claim that the copy is an AOAC method. This situation has not arisen, though. There usually is not enough information given about critical reagents such as DNA probes or antibodies to allow someone to easily replicate a method. AOAC Research Institute PTM approvals are specific to the individual commercial kit and annual reviews ensure that if reagent sources change, that a method modification study is performed to demonstrate equivalence.

7. Physical state of the cells
   In the ideal case, validation studies would only use naturally contaminated samples. However, these are rarely available, especially low-moisture foods contaminated with *Salmonella*. So, in the inoculated studies, the analyst stresses the inoculum and mixes it into the matrix so as to simulate conditions of natural contamination. For low-moisture foods, the model is to use a lyophilized cell pellet as the inoculum, mix it into the food, and allow the food to sit for 14 days to "stabilize". An MPN determination is then done to estimate the contamination level. For frozen foods, the food is thawed, inoculated with a culture dilution, and then re-frozen for 3 days before testing. In some cases, models of heat or preservative injury might be appropriate. For high-moisture refrigerated foods (e.g., raw or cooked chicken), the food is inoculated with a culture dilution and then refrigerated for 3 days before testing. There may be little or no injury in this case, but the thinking is that this realistically simulates the natural state of pathogen contamination of this type of product. No change to these procedures is currently recommended by the BPMM task force.

8. Phenotype vs. genotype
   Differences between molecular-based methods and immunological methods must be taken into consideration in method development and the design of validation studies. While molecular methods do not require expression of protein products, immunological methods must ensure that expression occurs at sufficient levels for detection to occur. Factors such as matrix, enrichment media, and enrichment temperature can potentially influence protein expression.

9. Sporulation
   No comments