

AOAC Review of Biotxin Laboratory Methods

Two methods have cleared the AOAC Task Force on Marine and Freshwater Toxins and are now being evaluated by AOAC for official status. These are the "ASP Direct cELISA" by Biosense Laboratories AS of Norway and a precolumn oxidation LC method for PSP toxins by Lawrence et al. of Health Canada.

Both methods are intended for the laboratory rather than the field and (once they attain official status) will supply alternatives to existing official methods.

The Biosense method is a proprietary test kit based on Enzyme-Linked Immunoassay (ELISA) - This method uses the specificity of antibodies to detect domoic acid and uses, in addition to the kit, a colorimetric microplate reader and allows the parallel analysis of large numbers of samples.

The method by Lawrence et al is a nonproprietary method using a liquid chromatograph and fluorescence detector: It differs from other LC methods for the PSP toxins in that it does not require the use of problematic post-column reaction systems or expensive mass-selective (MS) detectors. Toxins are first chemically oxidized to fluorescent products and then detected post-separation.

The response from CODEX has been positive regarding these methods/AOAC status.

The Task Force uses the following criteria in approving methods for AOAC evaluation.

Presidential Task Force on Marine and Freshwater Toxins
Analytical Methods Criteria for Prioritization and Selection

TO PRIORITIZE ORDER IN WHICH TOXINS/METHODS SHOULD BE ADDRESSED BY TASK FORCE

Need for Method - Demonstrated human illnesses due to toxin(s), with higher priority given for toxins known to cause human fatalities. Other impacts can include extensive economic damage and economic/cultural impact on subsistence groups. The method satisfies a recognized (public health related) need, for example it is given higher priority if there is no other method of analysis for toxins known to impact human health. Method may be needed to be able to fulfill national, regional, or international regulations, and limits exist which cannot be easily enforced. Higher priority is given to detection of toxins with greater potential for bioterrorist acts against the food and water supply.

Priorities of Outside Groups - The priority level of the method or method area is increased when International and/or influential groups (CODEX, UNESCO, FAO, EU, FDA, etc) have published documents expressing priority need for methodology in this toxin category.

Animal Assays/Legality Issues - Provides alternative to animal bioassay.

Full AOAC Method Performance Interlaboratory Study Exists - This is a special case, where higher priority can be given (still considering the criteria above) in that the results of an interlaboratory study are known. However the method must meet basic AOAC OMA criteria (this consideration is a special case at this point in time, June 2004, given current existence of such studies and also small number of approved AOAC methods for marine and freshwater toxins)

Presidential Task Force on Marine and Freshwater Toxins **Analytical Methods Criteria for Prioritization and Selection**

TO SELECT MOST APPROPRIATE METHODS FOR VALIDATION AND GUIDE METHODS DEVELOPMENT

Method Performance

- A. Method response to analyte can be related to toxin levels or activities of interest for toxin forms known to impact public health. Thus, the specificity desired is based on toxin class and known toxicity of individual forms and the method responds to toxin forms within a chemical class that make a significant contribution to overall toxicity. For assays (methods responding to total binding, biochemical or biological activity, etc.) the response parallels the overall toxicity.
- B. *Dynamic Range* of response, linear or otherwise, is adequate for purpose of clearly determining if analyte(s)/activity are above or below the level of interest.
- C. *Determination Limit* allows detection of total concentrations of currently known, impact-significant toxin forms or activities below action level (2x below [*or lower if appropriate*] for screening, 5x for quantitation).
- D. *Recovery* studies conducted in the sample matrix of interest. If available, naturally contaminated samples (analyzed by independent methodology) or analysis of Certified Reference Materials has been used to demonstrate adequate selectivity in the applicable sample matrix.
- E. *AOAC Single Laboratory Validation (SLV)* is preferred. Equivalent data accepted if it satisfies requirements for AOAC SLV study. Repeatability precision is acceptable as per SLV standards.
- F. *Ruggedness* - Critical (least rugged) parameters have been identified via ruggedness testing and are taken into account in method procedure, cautions, etc.
- G. *Standards* - Accurate calibration standards, and ideally, certified reference materials (CRMs), are readily available for implementation of the method as well as for routine validation and performance testing. Since calibration standards may not be available for all toxin forms in a group, the establishment and evaluation of relative response factors must be documented.

Practicality

Throughput meets program needs of stakeholders, and instrumentation, standards, techniques, etc. within budget or expertise limitations of stakeholder laboratories. Results are in units that allow use with action levels. If the method is proprietary, is that problematic in terms of availability of materials, continuity of method performance (e.g., reproducible production of antibodies), predicting performance in untested sample matrices, or in knowing critical parameters impacting ruggedness?