Interstate Shellfish Sanitation Conference
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M E M O R A N D U M

TO: Laboratory Methods Review Committee Members

FROM: Ken B. Moore, Executive Director Ken B. Moore

DATE: February 10, 2004

RE: 2004-2005 Committee Charges

This letter is to confirm your appointment by the Conference Chairman to the 2004-2005 Laboratory Methods Review Committee. At the August 2003 ISSC Biennial Meeting in Portland, Oregon I requested the Laboratory Methods Review Committee review information developed by D. W. Cook, FDA/GCL, related to use of alternative methods for identification of *Vv* in conjunction with MPN enumeration of *Vv* in molluscan shellfish. Initially I failed to make the Committee aware that the document was being offered as a possible solution because several laboratories had indicated concern regarding availability of supplies for the EIA procedure of Tamplin, et al. The D. W. Cook document, while not intended as a method approval request, was viewed by the Laboratory Methods Review Committee as such. The Committee advised the Executive Board that the only methods approved for confirmation of *Vv* in molluscan shellfish are the BAM-MPN-Biochemical and BAM MPN-EIA. The ISSC Executive Board, concerned for the status of previous and ongoing PHT validation studies using other methods as an alternative to the EIA, provided interim approval for gene probe technology. This decision was not supported by some members of the Laboratory Methods Review Committee. The decision of the Executive Board represents an acknowledgement by the Executive Board of a need to better understand the methodologies that are presently being used in laboratories that are conducting *Vv* analysis. The ISSC will establish a work group to assess the use of alternate technologies in laboratories conducting bacteriological analysis for the National Shellfish Sanitation Program (NSSP) purposes. This group will review the 1995 and 1998 editions of the US FDA Bacteriological Analytical Manual (BAM) to identify the status of various technologies prior to ISSC adoption of procedures for incorporation of laboratory methods into the NSSP. The work group will be requested to make recommendations to the Executive Board regarding the status of currently used methods and the need for conference review of new methods. The Laboratory Methods Review Committee will be requested to review any new methods that are submitted as proposals as a result of the assessment.

Additionally, the Committee is requested to reexamine the Jellett Rapid Test for PSP, prior to the 2005 ISSC Biennial Meeting, and provide a status report regarding the use of this method.

Thank you for your interest and support of the ISSC and I look forward to working with you. You will be contacted soon by your Subcommittee Chairman. If you are unable to accept this appointment, please contact me at 803-788-7559 or by e-mail at issc@issc.org.

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Methods for *Vibrio vulnificus*

Background:

The 1999 Model Ordinance, Chapter X states that the method for determining the end point criteria for post-harvest processed shellfish shall be the "*Vibrio vulnificus* FDA approved EIA procedure of Tamplin, et al. described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th edition, 1992". This method uses a monoclonal antibody specific for *V. vulnificus* for identification of isolates. The monoclonal antibody specific for *V. vulnificus* is no longer commercially available. Therefore, it is imperative that the ISSC approve alternate procedures for the identification of *V. vulnificus* isolates.

The 7th (1992), 8th (1995) and the 2001 revised electronic version of the 8th edition of the BAM (http://vm.cfsan.fda.gov/~ebam/bam-toc.html) permit the use of biochemical testing as aids to identification of suspect *V. vulnificus* isolates. Rapid biochemical test strips (e.g. API-20E) are recognized as an alternate to conventional tube format for biochemical tests. **Therefore, one option to correction the methodology problem would be for the ISSC to adopt biochemical testing as an alternative to the use of the EIA procedure identification of isolates.**

The 8th (1995) edition of the BAM and the 2001 revised electronic version both recognize a gene probe method for detection of cytotoxin-hemolysin gene of *V. vulnificus* which may be used as an additional identification procedure for *V. vulnificus*. This procedure which uses a non-radioactive probe was published by Wright et al., 1993, Appl. Environ. Microbiol. 59:541-546. More recent studies (DePaola, et al. 1997, Journal of Microbiological Methods 29: 115-120) reported that there was >90% agreement in the identity of isolates as being *V. vulnificus* when tested by both the gene probe and EIA procedures. This publication also compared results of the FDA-BAM procedure and a direct plating gene probe procedure for *V. vulnificus* in both oysters at harvest and market oysters finding that the direct plating gene probe method was an acceptable alternative to the BAM-EIA procedure. The American Public Health Association, Compendium of Methods for the Microbiological Examination of Foods, 4th edition, 2001, chapter 40, describes the use of the direct plating gene probe for enumerating *V. vulnificus*. Chapter 9 of the BAM is currently being revised and will contain the direct plating gene probe method for *V. vulnificus*. **A second option to correction the methodology problem would be for the ISSC to adopt the use of a gene probe specific for *V. vulnificus* as an alternative to the EIA procedure for identification of isolates.**

Another procedure for confirming the identity of isolates as *V. vulnificus* is by the use of Polymerase Chain Reaction (PCR). Detailed procedures for PCR detection of *V. vulnificus* are available in the 8th (1995) edition of the BAM, the 2001 revised electronic version of the BAM and the Compendium of Methods for the Microbiological Examination of Foods, 4th edition, 2001. **A third option to correction the methodology problem would be for the ISSC to adopt the use of a PCR procedure as an alternative to the EIA for identification of isolates.**

It is imperative that shellfish that have received post-harvest treatment be analyzed by a MPN procedure to permit detection of low densities of surviving *V. vulnificus*. Thus, biochemical, gene probe tests, or PCR could be used to identify the isolates. Unfortunately, neither the Compendium nor the revised BAM provides a descriptive methodology for using a gene probe

identification of *V. vulnificus* in conjunction with MPN enumeration. The following shows how the gene probe would replace EIA step for identification of isolates.

1. Process shellfish as stated in the BAM, (8th edition, 2001 revision) Chapter 9, Section D.2.a through d. This includes preparing the sample, inoculating into tubes of APW for MPN, overnight incubation, streaking each tube onto mCPC medium, and overnight incubation.

2. From each mCPC plate, transfer 3 typical (as described in the BAM) colonies into individual wells of 96-well plate containing 100 µl of APW per well. (Records must be kept to identify which MPN tube each colony originated from.)

3. Incubate plate for 3-4 hours at 35°C.

4. Use a 48-prong replicator (Bokel Industries, Feasterville, PA) to inoculate all wells from each half of the 96-well plate onto the surface of separate plate of T1N1 agar.

5. Incubate the plate overnight at 35°C

6. Prepare colony lifts, lyse cells, and probe with *V. vulnificus* (vvhA) gene probe as described in Compendium of Methods for the Microbiological Examination of Foods, 4th edition.

7. Correlate results of gene probe to MPN tubes. If any of the three colonies originating from an MPN tube are positive, that MPN tube would be scored as positive.

Recommendation for change in Model Ordinance:

1. Shellfish that have undergone post-harvest treatment shall be tested for *V. vulnificus* by the BAM-MPN (8th edition, 2001 revision) procedure with the use of the EIA procedure, biochemical test, the vvhA gene probe or PCR procedures to verify the identification of suspect isolates.

2. To determine *V. vulnificus* densities in shellfish that have not received post-harvest treatment, use procedures listed in (1) above or the direct plating gene-probe procedure (APHA, Compendium of Methods for the Microbiological Examination of Foods, 4th edition).

(This change would permit the use of any of the methods in the BAM or Compendium to be used to confirm identity of isolates as *V. vulnificus*.)

D.W. Cook, FDA/GCSL, 10/24/02