U.S. Department of Agriculture, Food Safety and Inspection Service Office of Public Health and Science, Laboratory QA Division

SOP No: PEPRL 0007. 10		Page 1 of 7
Title: Program Requirements for Recognized Laboratories		
Revision: 10	Replaces: PEPRL 0007.09	Effective 03/05/13

1. Purpose:

The USDA, FSIS PEPRLab Program is a program for laboratories performing *Salmonella* analysis on official surveillance samples of pasteurized egg product. This procedure, along with all requirements found in PEPRL F-0007 The *Salmonella* Laboratory Review Checklist and in PEPRL F-0008 The Salmonella Laboratory Self-Assessment Checklist, establishes the requirements that must be followed by all recognized laboratories in the PEPRLab Program.

2. Scope:

This procedure shall apply to all recognized laboratories in the PEPRLab Program.

3. References:

3.1 Ewing, W. H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae, 4th Edition. Elsevier Science Publishing Co., Inc., New York.

3.2 FDA's Bacteriological Analytical Manual (BAM) online http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm

3.3 Horowitz, William. (ed.) 2000. Official methods of analysis of AOAC International, 18th Edition. AOAC International Inc., Gaithersburg, MD 20877. Official Method 967.27.

3.4 Salmonella Surveillance Program for Liquid and Frozen Egg Products, FSIS Directive 10,230.4 (08/06/96).

3.5 Letter to Recognized Laboratory (August 14, 1995) from Isaac G. Sterling, (Former) Recognized Laboratory Coordinator, AMS, Technical Services Branch.

3.6 Letter to Supervisory Analysts, Recognized Egg Product Testing Laboratories (February 19, 1997) from Stanley S. Green, (Retired) Program Coordinator, PEPRLab Program.

3.7 Letter to Supervisory Analysts, FSIS Recognized Egg Testing Laboratories (October 16, 1997) from Stanley S. Green, (Retired) Program Coordinator, PEPRLab Program.

3.8 Acceptance Letter to New Laboratory (May 25, 1999) from Parmesh K. Saini, (Former) Project Manager, PEPRLab Program.

3.9 Memorandum to Supervisory Analysts, Recognized Egg Product Testing Laboratories (August 19, 1999), Subject: Availability of an Additional Cultural Method for *Salmonella* Testing of Pasteurized Egg Products from Parmesh K. Saini, (Former) Project Manager and Gerri M. Ransom, (Former) Program Coordinator, PEPRLab Program.

3.10 E-mail correspondence to Parmesh Saini, Staff Officer, USDA, FSIS (January 31, 2000), Subject: Test Lot – Surveillance Samples from Roger Glasshoff, USDA Technical Service Center.

3.11 Memorandum to Supervisory Analysts, Recognized Laboratories (February 7, 2000), Subject: Shipping Suspect *Salmonella* Isolates to another Laboratory for Confirmation from Parmesh K. Saini, (Former) Project Manager, PEPRLab Program

3.12 Letter to Recognized Laboratory (June 30, 2000) from Parmesh K. Saini, (Former) Project Manager, PEPRLab Program.

4. Specific Procedure(s):

4.1 **Each laboratory in the program must have a USDA, FSIS inspected egg-breaking or egg product plant as an ongoing client for whom they routinely perform mandatory USDA testing of egg product surveillance samples for the presence of *Salmonella***. The client has to submit surveillance samples to the laboratory on a regular basis within every 90 days.

4.1.1 Exemptions:

4.1.1.1 The analytical laboratories of USDA-AMS are exempted from this requirement, because they are engaged in the evaluation of adherence to purchase specifications for government buying programs, which include a zero tolerance for *Salmonella* in egg products purchased for school lunch programs.

4.1.1.2 Other Federal and State Laboratories may be exempted on a case-by-case basis, if they can establish that such an exemption will enhance the program's overall mission of ensuring the safety of the nation's pasteurized egg products.

4.1.2 Private commercial laboratories must report results on official surveillance samples expeditiously to the egg products firm. It is the responsibility of the egg products firm to make the test results (commercial or in-house), together with the name, location and PEPRLab number of the testing lab(s), available to the Salmonella Surveillance Program employee.

4.1.3 Laboratories must notify the program manager immediately of any addition, change, or loss of client.

4.1.4 Any recognized laboratory that loses its only remaining client **must have a new client in place within 90 days, or they will be dismissed from the program**.

4.1.5 It is the laboratory's responsibility to acquire replacement surveillance samples if there is any question concerning the integrity or identity of the samples initially submitted by the plant.

4.2 **Any recognized laboratory that does <u>not</u> use a rapid screening method in their testing program must use one of the following three cultural methods as their primary protocol for egg product analysis:**

4.2.1 AMS – Laboratory Methods for Egg Products – Section I (1993 revision) and Section VII (1994 revision).

4.2.2 FSIS MLG online, Chapter 4 – Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products.

4.2.3 FDA BAM online, Chapter 5 – *Salmonella*.

4.3 **A recognized laboratory may use a rapid screening method in their *Salmonella* testing program for FSIS official egg product surveillance samples provided it uses one of the following:**

1) the FSIS MLG rapid screening and confirmation methods, as written, **or**

2) a rapid screening method that is used in accordance with the manufacturer's instructions (including their enrichment scheme) that meets the requirements specified in sections 4.3.1 and 4.3.2 below. Documentation must be kept on file demonstrating that these requirements have been met. (It is suggested that the documentation on these two steps be obtained from the test manufacturer, and that each laboratory makes sure that the egg product types they routinely test are included in the study.

3) a rapid screen method that is incorporated into one of the approved culture methods (including the enrichment scheme) that meets the requirements specified in sections 4.3.1 and 4.3.2 below. Documentation must be kept on file demonstrating that these requirements have been met.

4.3.1 **The rapid method must be an approved Official Method of Analysis of the AOAC INTERNATIONAL (AOAC OMA) or an approved Performance Tested Method of the AOAC Research Institute (AOAC-RI PTM), validated for egg products, and capable of detecting both motile and non-motile *Salmonella*.**

4.3.2 **An additional comparative study of the rapid test with one of the 3 cultural methods listed in section 4.2 must be completed.** This comparative study usually is performed by the test manufacturer on a variety of *Salmonella*-inoculated egg products.

4.3.2.1 The method under study must be found to be equivalent to or better than the cultural method. All *Salmonella* positive results must be confirmed.

4.3.2.2 The data from this study must demonstrate that the test performance characteristics (sensitivity, specificity, efficiency, false-positive rate, false-negative rate, and detection limit) from the

analysis of the inoculated egg products agree with those published in the full AOAC collaborative study.

4.3.3 **A recognized laboratory that plans to start using a rapid screening method or to switch to a different rapid screening method must <u>also</u> do additional testing to demonstrate competence in performing the alternative *Salmonella* screening method**. A recognized laboratory wishing to use an acceptable rapid screening method on official egg product surveillance samples must first run a comparison study between that rapid screening method and one of the three cultural methods listed in section 4.2. This must be done by testing, with each method, at least twenty samples of egg product, one-half of which must be uninoculated (negative) and one-half of which must be inoculated with *Salmonella* 10 – 15 CFU/g in a 100 g sample. The inoculated samples used in the study may be identically prepared or split samples and must include the egg product types routinely tested by the laboratory. (i.e. 10 liquid and 10 dried samples, if both types are tested by the laboratory.) The laboratory must demonstrate at least a 95% agreement between the results of the two methods tested.

4.4 **Each presumptive positive sample must be confirmed using one of the three cultural methods listed in section 4.2 or according to the rapid screen method manufacturer's insert specifications.** Cultural confirmation must include the testing and reporting of the biochemical and serological [polyvalent somatic (O) and polyvalent flagellar (H) antisera] identification of the *Salmonella* isolate. See section 3 (references) for further details on biochemical confirmation.

4.4.1 If a rapid/miniaturized biochemical test system is used, it must be an **approved AOAC OMA or an approved AOAC-RI PTM.**

4.5 Regardless of the method used:

4.5.1 **The laboratory <u>must</u> run uninoculated media controls and method prescribed positive *Salmonella* culture controls along with each *Salmonella* analysis of surveillance samples.**

4.5.1.1 The controls must start with the pre-enrichment steps and be carried along with the samples through all steps, including the screen tests and any confirmation steps.

4.5.1.2 If no samples are positive in the test, the controls may be terminated at the same point as the samples.

4.5.2 **FSIS official egg product sample size for analysis shall be at least 100 grams of egg product.** The preferable sample size for FSIS official surveillance samples is 100 grams. (The sample may be analyzed in two 50 gram portions or four 25 gram portions, if necessary.)

4.5.3 The **ratio of egg sample to preenrichment medium** shall be maintained at **1:10.** (For example: 100g of egg product plus 900 ml of preenrichment medium.)

4.5.4 A laboratory must specify which method will be used for each (program) client, and they shall be audited to this.

4.5.4.1 The laboratory shall notify the program manager immediately of any change in method.

4.6 **It is required that any laboratory wishing to ship suspect *Salmonella* isolates (including isolates from check samples) to another lab for confirmation must use only another recognized laboratory (in the PEPRLab Program) and must follow these steps:**

4.6.1 The tetrathionate, selenite cystine, Rappaport-Vassiliadis, or screen test broth (M broth or GN broth or other) shall be inoculated onto the differential selective agar plates as described in the approved method. (<u>This step must be performed at the testing laboratory initiating analysis of the sample</u>.)

4.6.2 After incubation of the plates, suspect colonies shall be picked onto triple sugar iron (TSI) and lysine iron agar (LIA) slants.

4.6.3 <u>After incubation of the slants</u>, the TSI and LIA slants are shipped to the confirming laboratory.

4.6.4 The testing laboratory initiating analysis shall retain backup cultures and shall maintain complete records of the analysis, including records of the work performed by the confirming laboratory, as well as the confirming laboratory's name and PEPRLab number.

4.6.5 PT samples must be analyzed by the same laboratory(s) as the official surveillance samples of pasteurized egg products.

4.7 **The unique PEPRLab number issued to each laboratory <u>must be used on all official correspondence</u> pertaining to the PEPRLab Program.**

4.8 **The person in charge of microbiology shall have a baccalaureate degree in biology, chemistry, microbiology, food technology, medical technology, or other relevant science with at least 12 semester hours of course work in microbiology and/or at least 4 years of experience working in a public health, medical, food, or other related laboratory.**

4.9 **All laboratory personnel shall be trained in microbiological laboratory procedures and systems, documentation, equipment operation and maintenance, and laboratory safety.**

4.9.1 Written documentation shall be kept on file demonstrating that these requirements have been met. The written documentation shall provide an ongoing assessment of analytical performance and competency by

individual analysts within the laboratory through a continuous record of training and proficiency test (PT) results.

4.10 **On a yearly basis, the PEPRLab program manager shall request that each laboratory submit an Information Update Request Form (PEPRL F-0003). This form must be completed and returned within two weeks of receipt of the form. Additionally, laboratories must notify the program manager of any change in client, method(s), analytical or management personnel, or address within 30 calendar days of the occurrence.**

4.11 **On a yearly basis, a recognized laboratory must successfully analyze all PT samples submitted through the program.** Successful analysis of PT samples is defined as correctly identifying at least 90% of the egg PT samples for every two consecutive PT events.

4.11.1 Five liquid egg samples are sent out on a semiannual basis to each of the member laboratories by a contracted supplier.

4.11.1.1 These PT samples shall be used for the evaluation of each laboratory's proficiency in the analysis of egg products for the presence of *Salmonella*. Laboratories must use the same confirmatory laboratory and methods that are used to analyze official egg surveillance samples.

4.11.2 Any laboratory that fails to meet these requirements will be placed on probation until filing an acceptable corrective action response, and successfully analyzing a double set of PT samples (90% correct identification). Failure to successfully analyze the double set of PT samples will result in immediate dismissal from the program.

4.11.3 While a dismissed laboratory may reapply to the program following the entry guidelines defined in SOP PEPRL 0004, reentry in the program shall be decided on a case by case basis.

4.11.4 A dismissed laboratory must have their client send samples to another recognized laboratory in the PEPRLab Program.

4.11.5 No charges for PT samples are incurred by a recognized laboratory, unless a deficiency occurs in which the laboratory is at fault (e.g. mishandling the samples after receipt, not starting them on time, losing the data result sheet after analysis, etc.) and the result requires that additional samples be requested.

4.11.5.1 In that case the laboratory must pay the cost of the replacement PT sample set, as well as the cost of shipping.

4.11.5.2 Any charges incurred by a recognized laboratory will be invoiced directly by the contractor.

4.12 **Each laboratory, with the exception of the exempted laboratories listed in Section 4.1.1, must be found acceptable in audits, which may be performed on an annual basis, but no less often than once every 3 years**. See SOP PEPRL 0005. All items/issues listed in *Salmonella* Laboratory Review Checklist PEPRL F-0007 and Self - Assessment Checklist PEPRL F-0008, and Program Requirements for Recognized Laboratory PEPRL 0007 are program requirements, and must be followed. Any deviations from the program requirements found in an audit will result in audit deficiencies.

4.12.1 The laboratory must provide written documentation of the corrective actions taken in response to any deficiencies listed in the audit report. The corrective action response must include objective evidence supporting each of the corrective actions taken and must be submitted within 30 days of the report receipt date in order to remain in the program.

4.12.2 A follow up request for additional objective evidence and/or a follow up audit may be conducted at the discretion of the program manager to ensure continued compliance with program requirements for laboratories cited with major or multiple deficiencies during their previous audit.