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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

**SALMONELLA LABORATORY SELF-ASSESSMENT CHECKLIST**

LABORATORY NAME: \_\_\_\_\_

LABORATORY DIRECTOR: \_\_\_\_\_

LABORATORY REPRESENTATIVE(S) AT REVIEW: \_\_\_\_\_

\_\_\_\_\_

REVIEWER: \_\_\_\_\_

DATE OF REVIEW: \_\_\_\_\_

**OPENING QUESTIONS:**

1. Who are your current clients?

Client: \_\_\_\_\_ Establishment No. \_\_\_\_\_

Client: \_\_\_\_\_ Establishment No. \_\_\_\_\_

2. Is this facility an in-plant laboratory?

3. On average, how many *Salmonella* tests are conducted per week? \_\_\_\_\_

4. How many of these tests are on USDA Official Surveillance Samples? \_\_\_\_\_

5. When was the last time a pasteurized egg product sample was found to be positive for *Salmonella*? \_\_\_\_\_

6. How many *Salmonella* positive pasteurized egg product samples have been found in the last 3 years? \_\_\_\_\_

7. How soon and to whom were these reported? \_\_\_\_\_

\_\_\_\_\_

8. List all personnel involved in the *Salmonella* testing. \_\_\_\_\_

\_\_\_\_\_

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

**TABLE OF CONTENTS**

Opening Questions	Page 1
Table of Contents	Page 2
Section A: Personnel Requirements	Page 3
Section B: Physical Facilities	Page 3
Section C: Sample Receipt and Handling	Page 4
Section D: Quality Assurance	Page 6
Section E: Media and Reagents	Page 10
Section F: Analytical Procedures Manual	Page 13
Section G: Procedures and Methods	Page 15
AMS Method	Page 16
FSIS Method	Page 17
FDA Method	Page 18
Procedures and Methods (continued)	Page 19
Section H: Safety	Page 21

U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

**A. PERSONNEL REQUIREMENTS**

- |    |  |     |     |     |
|----|--|-----|-----|-----|
| 1. | Does the person in charge of microbiology 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? | Yes | No  | N/A |
| 2. | Are there training/education/experience records available for each analyst?  | Yes | No  | N/A |
| 3. | Is there a formal training program for employees working in microbiology that includes instruction in safety, technical procedures, and use of equipment?  | Yes | No  | N/A |
| 4. | Is there a record kept of this formal training?<br>N/A   |     | Yes | No  |

**B. PHYSICAL FACILITIES**

A laboratory should have sufficient work and storage space and the facilities to handle the overall workload in order to ensure the quality of work, and safety of the employees.

- |    |   |     |    |     |
|----|---|-----|----|-----|
| 1. | Are floors, benches, and storerooms clean, free of clutter, dust free, and well maintained? | Yes | No | N/A |
| 2. | Are the following facilities adequate:  |     |    |     |
| a. | Sinks?  | Yes | No | N/A |
| b. | Lighting?   | Yes | No | N/A |
| c. | Gas outlets/Bacti-cinerator?  | Yes | No | N/A |
| d. | Electrical outlets?   | Yes | No | N/A |
| e. | Incubator capacity?   | Yes | No | N/A |

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

f.	Refrigerated storage space?	Yes	No	N/A
g.	Ventilation?	Yes	No	N/A
3.	Is there sufficient bench space for each analyst?	Yes	No	N/A
4.	Are bench tops made of impervious materials?	Yes	No	N/A
5.	Is the media preparation, glassware washing area separate from the analytical area?	Yes	No	N/A
6.	Is unrelated traffic discouraged in the work area, and is the laboratory locked when analysts are not present?	Yes	No	N/A
7.	Are samples that are stored at room temperature, stored in sealed containers to prevent pests from infesting the laboratory?	Yes	No	N/A
8.	Is there a pest control system in place for the laboratory?	Yes	No	N/A

**C. SAMPLE RECEIPT AND HANDLING**

Samples must be submitted to the laboratory in a condition that does not compromise the quality and validity of analytical results, and must be handled after receipt in the laboratory in a manner to maintain sample integrity.

l.	Are samples inspected upon receipt in the laboratory for:			
a.	Leakage?	Yes	No	N/A
b.	Thawed frozen samples?	Yes	No	N/A
c.	Unsealed or ruptured containers?	Yes	No	N/A
d.	Spoilage?	Yes	No	N/A
e.	Evidence of tampering?	Yes	No	N/A

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

- |    |   |     |    |     |
|----|---|-----|----|-----|
| 2. | Are all samples (including rejected samples) recorded in a login system book, on worksheets, by computer, or in another permanent, accessible format? | Yes | No | N/A |
| 3. | Are unacceptable samples rejected for analysis and is the condition recorded in login system book, computer, etc?                                     | Yes | No | N/A |
| 4. | If yes, are acceptable samples resubmitted?   | Yes | No | N/A |
| 5. | Does sample information include at a minimum:   |     |    |     |
|    | a. Lot number?  | Yes | No | N/A |
|    | b. Date of collection?  | Yes | No | N/A |
|    | c. Plant name and/or number?  | Yes | No | N/A |
|    | d. Type of analysis requested?  | Yes | No | N/A |
|    | e. Type of product/state of product?  | Yes | No | N/A |
|    | f. Date of receipt?   | Yes | No | N/A |
|    | g. Condition upon receipt?  | Yes | No | N/A |
| 6. | Are liquid samples either analyzed on the same day received or refrigerated at 2.0 to 8.0°C until analyzed?   | Yes | No | N/A |
| 7. | Is the maximum turnaround time for sample analyses:   |     |    |     |
|    | a. 4 to 5 days for negatives (cultural isolation method)?   | Yes | No | N/A |
|    | b. 5 to 7 days for positives (cultural isolation method)?   | Yes | No | N/A |
|    | c. 2 to 3 days for negatives using a rapid screening procedure?   | Yes | No | N/A |
| 8. | Are frozen samples either rapidly thawed in a water bath  |     |    |     |

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

- (preferably with agitation) at less than 45°C until the slush ice stage (for no longer than 30 minutes) or thawed at refrigerator temperatures (2.0 to 8.0°C) for no longer than 18 hours in the original container? Yes No N/A
9. Are thawed frozen samples analyzed immediately? Yes No N/A
10. Are dried egg samples analyzed upon receipt or stored at room temperature for no more than 24 hours? Yes No N/A
11. If analysis of dried egg samples is delayed more than 24 hours, are they refrigerated at 2.0 to 8.0°C? Yes No N/A
12. Are samples placed in appropriate storage after analysis and are negatives retained for at least one day after reporting and are positives retained for at least 30 days? Yes No N/A

**D. QUALITY ASSURANCE**

A written quality assurance program for the laboratory should be available, and the quality control records should be reviewed at least weekly by the supervisor. Proper care of laboratory instruments and equipment is essential for satisfactory performance of laboratory tests. Maintenance must be performed on a regular basis by trained individuals. Monitoring must be performed at stated intervals by laboratory personnel to assure on-going reliability.

1. Is there a written Quality Assurance Program? Yes No N/A
2. Is there documentation showing that records of procedure controls, instrument functions, scheduled maintenance, and equipment temperatures are reviewed at least weekly? Yes No N/A
3. Is there documentation showing that corrective action(s) were taken when controls were found to be unacceptable and/or when instruments were found to be non functioning or to have failed? Yes No N/A
4. Are quality control and maintenance records

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

- |     |   |     |    |     |
|-----|---|-----|----|-----|
|     | maintained for at least 3 years?  | Yes | No | N/A |
| 5.  | Is there a system for routinely reviewing the work to detect clerical or analytical errors, or unusual results?   | Yes | No | N/A |
| 6.  | Does the system provide for timely correction of errors?  | Yes | No | N/A |
| 7.  | Are laboratory results and analysts' worksheets retained for each sample including negative samples for a period of at least three years?   | Yes | No | N/A |
| 8.  | Are there records of internal reviews and, when indicated, corrective action(s) taken in response to unacceptable check-sample results?   | Yes | No | N/A |
| 9.  | Are thermometers checked for accuracy against a thermometric standard (National Institute of Standard and Technology/formerly National Bureau of Standards) before placing them in service? | Yes | No | N/A |
|     | a. Are thermometers calibrated annually?  | Yes | No | N/A |
|     | b. Are correction factors listed on each thermometer?   | Yes | No | N/A |
|     | c. Is the NIST traceable thermometer sent in for calibration at least every 5 years?  | Yes | No | N/A |
| 10. | Are mechanical pipetting devices calibrated at least semi-annually to check accuracy of delivery?   | Yes | No | N/A |
| 11. | Is there a scheduled, written preventative maintenance program for laboratory equipment and instruments?  | Yes | No | N/A |
| 12. | Does the preventative maintenance program include the following:  |     |    |     |
|     | <b>I. AUTOCLAVES:</b>   |     |    |     |
|     | a. Are acceptable temperature ranges defined for autoclaves?  | Yes | No | N/A |
|     | b. Are there recording thermometers, calibrated dials,  |     |    |     |

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

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| or other recording devices present on autoclaves?   | Yes | No | N/A |
| c. Are temperatures checked and recorded at each use and is there documentation of corrective action for out-of-range results?                            | Yes | No | N/A |
| d. Are autoclaves monitored with biological indicators at least monthly and are they monitored each time used with a physical indicator (indicator tape)? | Yes | No | N/A |
| <b>II. WATERBATHS:</b>  |     |    |     |
| a. Are thermometers suspended in distilled water?   | Yes | No | N/A |
| b. Are acceptable temperature ranges defined and available for waterbaths?  | Yes | No | N/A |
| c. Are temperatures checked and recorded at least daily, and is there documentation of corrective action for out-of-range results?                        | Yes | No | N/A |
| d. Are water baths clean and free of debris, and is the water changed regularly?  | Yes | No | N/A |
| <b>III. INCUBATORS:</b>   |     |    |     |
| a. Are thermometers suspended in an appropriate liquid such as sterile glycerin, distilled water or other acceptable medium?                              | Yes | No | N/A |
| b. Are acceptable temperature ranges defined and available for each incubator?  | Yes | No | N/A |
| c. Are temperatures checked and recorded at least daily, and is there documentation of corrective action for out-of-range temperatures?                   | Yes | No | N/A |
| <b>IV. REFRIGERATORS/FREEZERS:</b>  |     |    |     |
| a. Are thermometers suspended in an appropriate   |     |    |     |



U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

liquid such as sterile glycerin, distilled water or other acceptable medium?  
 Yes No N/A

b. Are acceptable temperature ranges defined and available for refrigerators/freezers?  
 Yes No N/A

c. Are temperatures checked and recorded at least daily, and is there documentation of corrective action for out-of-range temperatures?  
 Yes No N/A

**V. SPECTROPHOTOMETERS AND PHOTOMETRIC READERS:**

a. Are manufacturer's operation requirements followed for the spectrophotometer and photometric reader?  
 Yes No N/A

b. Is the instrument calibrated according to manufacturer's requirements or kit manufacturer's requirements?  
 Yes No N/A

**VI. BALANCE:**

a. Is the balance checked with a certified set of weights at least weekly?  
 NOTE: A 2000 gram balance must have a sensitivity of 0.1 grams with a 200 gram load?  
 Yes No N/A

b. Is the balance checked annually by an authorized service representative using certified weights that are traceable to the National Institute of Standards and Technology?  
 Yes No N/A

**VII. pH METER:**

a. Are pH meters standardized with at least two appropriate standard buffer solutions covering the range of intended use prior to use and are the results recorded?  
 Yes No N/A

b. If pH readings are going to be taken intermittently throughout

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

- |   |     |    |     |
|---|-----|----|-----|
| the day, is the pH meter re-calibrated with fresh portion of buffers before each use?                 | Yes | No | N/A |
| c. Are pH meter electrodes checked each time they are used to see if they are filled and not cracked? | Yes | No | N/A |

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

**E. MEDIA AND REAGENTS**

All media, reagents, and chemicals must be prepared correctly, stored under appropriate conditions, and tested with reference organisms to assure satisfactory performance.

- |    |  |     |    |     |
|----|--|-----|----|-----|
| 1. | Are all purchased media, chemicals and solutions labeled with the date received and an expiration date?        | Yes | No | N/A |
| 2. | Are all in-house prepared media, reagents, and solutions labeled with the name of product and expiration date? | Yes | No | N/A |
| 3. | Do media records contain complete QC information for each batch, including pH, sterility, and productivity?    | Yes | No | N/A |

If pH paper strip is used for pH determination, the pH paper has to cover the pH range of use with pH gradation value  $\leq 0.2$  pH unit.

- |    |   |     |    |     |
|----|---|-----|----|-----|
| 4. | Are media, reagents, and/or solutions stored under appropriate conditions (i.e. refrigerated, away from daylight, in a cool or dry place and in appropriate laboratory containers)? | Yes | No | N/A |
|----|---|-----|----|-----|

Note: The shelf life of prepared media will vary. In general, the maximum shelf life of prepared culture media in sealed tubes or bottles is 3 months in the refrigerator (2 - 8°C), or up to 1 month at room temperature (18 - 23°C). Media in vented tubes may be stored for up to 4 weeks if refrigerated or 2 weeks at room temperature. Plating media may be stored in the refrigerator for a maximum of 10 weeks in air-tight bags or for a maximum of 2 weeks if the bags are unsealed.

- |    |   |     |    |     |
|----|---|-----|----|-----|
| 5. | Are outdated materials discarded?   | Yes | No | N/A |
| 6. | Is a sample of each batch of in-house prepared media checked for the ability to support growth (and for biochemical reactivity/selectivity, as appropriate to the media) by using reference organisms capable of evaluating pertinent characteristics of the media? | Yes | No | N/A |

List the *Salmonella* media QA cultures used: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

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|-----|--|-----|-----|-----|
| 7.  | Are reference organisms maintained under refrigeration on agar with at least monthly transfers, or by other appropriate methods?   | Yes | No  | N/A |
| 8.  | Is an uninoculated control of each medium used and run concurrently with the sample?   | Yes | No  | N/A |
| 9.  | Are all media in satisfactory condition upon visual examination (i.e. uncontaminated, hydrated, smooth, appropriate color and thickness) and results documented for each batch?  | Yes | No  | N/A |
| 10. | Are serological reagents tested with appropriate positive and negative controls?<br>(Note: This may include culture controls, commercially produced antigen, or kit controls.)   | Yes | No  | N/A |
| 11. | Are serological reagents refrigerated when not in use, inspected for clarity and color, and discarded when showing any turbidity, flocculation or color change?<br>N/A   |     | Yes | No  |
| 12. | Is Rappaport Vassiliadis broth (e.g. RV10, RVS, or RV Broth) prepared according to manufacturers instructions and autoclaved for 15 minutes at 115 or 116 °C (12 lbs)?<br>(NOTE: It is important not to overheat this medium.) | Yes | No  | N/A |
| 13. | Is tetrathionate broth prepared according to manufacturers instructions and heated to a boil?<br>(NOTE: It is important not to overheat this medium.)  | Yes | No  | N/A |
| 14. | Is only the basal medium of tetrathionate broth base stored?   | Yes | No  | N/A |
| 15. | Is the iodine - potassium iodide solution added to the tetrathionate broth base on the day of use?   | Yes | No  | N/A |
| 16. | Is selenite cystine broth prepared only by boiling, and is it used on the day of preparation?  | Yes | No  | N/A |
| 17. | Are Bismuth Sulfite Agar (BS) plates prepared, stored,   |     |     |     |

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

and incubated only as follows:

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|-----|--|-----|----|-----|
| a.  | Are BS plates prepared (20 to 25 ml/plate) from dehydrated media that is smooth, free-flowing, and has been properly stored?   | Yes | No | N/A |
| b.  | Are BS plates used on the day of preparation or no more than 1 day after preparation?  | Yes | No | N/A |
| 18. | Are double modified lysine iron agar (DMLIA) plates used within three weeks of preparation (for FSIS method)?  | Yes | No | N/A |
| 19. | In the preparation of XLT4 agar (FSIS METHOD), is one of the following used:   |     |    |     |
| a.  | XL Agar Base with Thiosulfate citrate and a 27 % solution (approximate) of the surfactant 7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt, formerly produced by Union Carbide under the tradename of Tergitol 4?      | Yes | No | N/A |
| b.  | XLT4 Agar Base with the XLT4 Agar Supplement (a 27 % solution (approximate) of the surfactant 7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt, formerly produced by Union Carbide under the tradename of Tergitol 4). | Yes | No | N/A |
| 20. | Does the laboratory have a distillation, ion exchange, filtration, or other system available for producing or purchasing water, free from toxic or nutritive substances, to be used in media or reagent preparation?             | Yes | No | N/A |
| 21. | Is the distilled water stored properly?  | Yes | No | N/A |
| 22. | Is the water system monitored at least monthly and/or is there a certificate of analysis for purchased distilled water to ensure that each meet the following criteria:  |     |    |     |
| a.  | conductivity (< 1.0 μSiemens) or resistivity (> 1 Megohm)?   | Yes | No | N/A |

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

<h2 style="margin: 0;">Pasteurized Egg Products Recognized Laboratory (PEPRLab) Program</h2>
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- |     |  |     |    |     |
|-----|--|-----|----|-----|
|     | b. bacteria (<1000 cfu /ml)?   | Yes | No | N/A |
| 23. | Are other media used for <i>Salmonella</i> testing of pasteurized egg products?<br>If so, list the media used: _____<br>_____<br>_____ | Yes | No | N/A |
| 24. | If so, are these media prepared and stored according to the manufacturer's instructions?   | Yes | No | N/A |

### F. ANALYTICAL PROCEDURES MANUAL

To assure consistent laboratory results, a procedures manual should be available at each work station and should contain all procedures performed in the laboratory. Procedures should be written in sufficient detail to enable the analyst(s) to perform tests without referring to other publications. **(NOTE: Manufacturers' package inserts with specific product use instructions may be used in addition to the manual, but cannot replace the procedures manual.)**

A recognized laboratory may use a rapid screening method in their testing program for FSIS official egg product surveillance samples only if that method is either an approved AOAC Official Method of Analysis of the AOAC INTERNATIONAL, validated for egg products, or the FSIS Rapid Screening Method as described in the MLG. All presumptive positives identified by rapid screening methods must be confirmed using one of the three accepted cultural methods listed below. Any recognized laboratory that does not 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:

1. AMS Laboratory Methods for Egg Products – Section I ('93 rev.) and Section VII ('94 rev.)  
Reference AOAC 967.26, 967.27, 978.24, 989.12, 991.13.
2. FSIS MLG online, Chapter 4.
3. FDA BAM online, Chapter 5.

- |    |   |     |    |     |
|----|---|-----|----|-----|
| 1. | Is an Analytical Procedures Manual available in the laboratory? | Yes | No | N/A |
| 2. | For <i>Salmonella</i> testing, does the manual contain:         |     |    |     |

U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

- |    |  |     |     |     |
|----|--|-----|-----|-----|
| a. | All of the procedures performed?   | Yes | No  | N/A |
| b. | Only approved or accepted procedures?  | Yes | No  | N/A |
| c. | Criteria for accepting or rejecting samples?   | Yes | No  | N/A |
| d. | A section on media and reagent preparation?  | Yes | No  | N/A |
| e. | Quality control procedures?  | Yes | No  | N/A |
| 3. | Does each procedure contain:   |     |     |     |
| a. | Step-by-step instructions?   | Yes | No  | N/A |
| b. | Sample handling/preservation?  | Yes | No  | N/A |
| c. | Expected reactions/results?  | Yes | No  | N/A |
| d. | Corrective actions to be taken when expected reactions/results are not observed?   | Yes | No  | N/A |
| e. | References?  | Yes | No  | N/A |
| 4. | Is the manual reviewed and updated annually?   | Yes | No  | N/A |
| 5. | Are changes in procedures approved and initialed by the supervisor?  | Yes | No  | N/A |
| 6. | Is there documentation to show that all analysts have read the procedures manual, including any revisions, and that only the most recent revision is being used? |     | Yes | No  |
|    | N/A  |     |     |     |

**G. PROCEDURES AND METHODS**

U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

Routine procedures for *Salmonella* detection permit recovery of small numbers of pathogens or debilitated organisms by pre-enrichment in lactose broth or buffered peptone water (BPW). Selective enrichment and plating procedures following that permit growth of *Salmonella* while limiting the growth of competing non-*Salmonella* organisms naturally present in food samples. Identification of an isolate as a member of the genus *Salmonella* depends on a combination of biochemical and serological parameters.

- |    |   |     |    |     |
|----|---|-----|----|-----|
| 1. | Is at least 100 g of sample tested for official surveillance samples?   | Yes | No | N/A |
| 2. | Is a positive control culture run along with all <i>Salmonella</i> tests through any rapid screening test and confirmation tests? | Yes | No | N/A |

List the *Salmonella* control culture(s) used: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

- |    |  |     |    |     |
|----|--|-----|----|-----|
| 3. | Is every tube and plate throughout the test procedure appropriately labeled?   | Yes | No | N/A |
| 4. | For each sample, are records maintained documenting each step of analysis for traceability? (i.e. analyst ID, media/kit/reagent lot number, incubation time and temperatures, equipment ID number, etc.) | Yes | No | N/A |
| 5. | List the cultural method used for analysis and/or confirmation of official surveillance samples. _____   |     |    |     |
| 6. | Is the laboratory using a rapid screening method that is either the FSIS MLG Method or an approved AOAC Official Method, validated for egg products?   | Yes | No | N/A |

If yes, list the method below with its AOAC reference number:

Rapid Screening Method: \_\_\_\_\_

AOAC Official Method Reference Number: \_\_\_\_\_

- |    |   |  |  |  |
|----|---|--|--|--|
| 7. | Prior to implementing a new rapid method, were parallel tests conducted using both the rapid and conventional |  |  |  |
|----|---|--|--|--|



U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

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|     | cultural methods and were the results documented?  | Yes | No | N/A |
| 8.  | In the parallel testing, did the methods show equivalency, agreeing at least 95 percent of the time?   | Yes | No | N/A |
| 9.  | Are all positive results that are obtained by rapid screening methods followed up by subculturing the sample and subsequently performing biochemical and serological identification of any <i>Salmonella</i> isolates? | Yes | No | N/A |
| 10. | Is the ratio of egg sample to preenrichment broth maintained at 1:10?  | Yes | No | N/A |

**Proceed to question #11 if your lab is using the AMS culture method. Go to question #12 if your lab is using the FSIS, MLG chapter 4 culture method. Go to question #13 if your lab is using the FDA, BAM chapter 5 culture method.**

11. **AMS Method – Laboratory Methods for Egg Products (Section I - 1993 rev.) and Section VII - 1994 rev.):**

- |    |  |     |    |     |
|----|--|-----|----|-----|
| a. | Is the pH of the lactose broth/egg mixtures adjusted to $6.8 \pm 0.2$ after being left for 1 hour at room temperature?   | Yes | No | N/A |
|    | List the method of pH testing used: _____  |     |    |     |
| b. | After $24 \pm 2$ hours incubation at $35^{\circ}\text{C}$ is the lactose broth subcultured by transferring 1 ml into 10 ml of selenite cystine broth and an additional 1 ml into 10 ml of tetrathionate broth?               | Yes | No | N/A |
| c. | After $24 \pm 2$ hours incubation at $35^{\circ}\text{C}$ are the selenite cystine and tetrathionate broths subcultured to selective differential agars, XLD, HE, and BS (or manufacturer's recommendation for rapid tests)? | Yes | No | N/A |
| d. | After $24 \pm 2$ hours incubation at $35^{\circ}\text{C}$ are up to three typical colonies (if available), characteristic of <i>Salmonella</i> species, selected from each differential agar plate as follows:               |     |    |     |

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

**XLD** – pink/red colonies with/without black centers or all black colonies (atypical strains may appear yellow with or without black centers)?

Yes No N/A

**HE** – blue/blue-green colonies with or without black centers or all-black colonies?

Yes No N/A

**BS** – brown, black, or grey colonies, usually with a metallic sheen and darkening of the surrounding media or occasionally green colonies?

Yes No N/A

e. Are all BS agar plates examined for typical or suspicious *Salmonella* colonies after 24 ± 2 hours incubation at 35°C and, if negative, again at 48 ± 2 hours incubation?

Yes No N/A

f. Go to question #14. (page 19)

**12. FSIS Method – Microbiology Laboratory Guidebook online (MLG), Chapter 4:**

a. After 20 – 24 hours of incubation at 35 ± 2°C, is the buffered peptone water-sample mixture subcultured by transferring 0.1 ml. into 10 ml of Rappaport Vassiliadis (RV) Broth and by transferring 0.5 ml into 10 ml of tetrathionate (TT) broth, and are these broths then incubated at 42 ± 0.5°C?

Yes No N/A

b. After 22 – 24 hours incubation at 42 ± 0.5°C are TT and RV broths subcultured to selective differential agars, BGS and either DMLIA or XLT4?

Yes No N/A

c. After 18 – 24 hours incubation at 35 ± 2°C are up to three typical colonies, (if available), characteristic of *Salmonella* species, selected from each differential agar plate as follows:

**BGS** – colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium? (On very crowded plates, look for colonies that give a tan appearance against a green background.)

Yes No N/A

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

**DMLIA** – purple colonies with or without black centers? (Since salmonellae typically decarboxylate lysine and ferment neither lactose nor sucrose, the color of the medium reverts to purple.)

Yes No N/A

**XLT4** – black colonies or red colonies with black centers? (The rim of the colony may still be yellow in 24 h; later it should turn red.)

Yes No N/A

d. Are all selective agar plates reincubated for an additional 24 ± 2 hours and are all initially negative plates, as well as those yielding non-confirmed *Salmonella* colonies from the initial selection reexamined before discarding?

Yes No N/A

e. Go to question #14. (page 17)

**13. FDA Method – Bacteriological Analytical Manual online (BAM), Chapter 5:**

a. Is lactose broth used for pre-enrichment of dry egg products?

Yes No N/A

b. Is TSB with ferrous sulfate (35 mg ferrous sulfate per 1000 ml TSB) used for pre-enrichment of liquid egg products?

Yes No N/A

c. Is the pH of the pre-enrichment broth/egg mixtures adjusted to 6.8 ± 0.2 after being left for 1 hour at room temperature?

Yes No N/A

List the method of pH testing used: \_\_\_\_\_

d. After 24 ± 2 hours incubation at 35°C is the lactose broth subcultured by transferring 0.1 ml into 10 ml of RV broth and an additional 1 ml into 10 ml of tetrathionate (TT) broth?

Yes No N/A

e. Is the RV broth incubated 24 h ± 2 h at 42 ± 0.2°C?

Yes No N/A

f. Is the TT broth incubated 24 h ± 2 h at 35 ± 2.0°C?

Yes No N/A

g. After 24 ± 2 hours incubation are the RV and TT broths

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

subcultured to selective differential agars, XLD, HE, and BS by streaking 10 µl from each broth onto each of the three selective differential agars? Yes No  
 N/A

h. After 24 ± 2 hours incubation at 35°C are at least 2 typical colonies (if available), characteristic of *Salmonella* species, picked to TSI and LIA slants from each differential agar plate as follows:

**XLD** – pink/red colonies with/without black centers or all black colonies (atypical strains may appear yellow with or without black centers)? Yes No N/A

**HE** – blue/blue-green colonies with or without black centers or all-black colonies? Yes No N/A

**BS** – brown, black, or grey colonies, usually with a metallic sheen and darkening of the surrounding media or occasionally green colonies? Yes No N/A

i. Are BS plates re-incubated an additional 24 ± 2 h and, if the original colonies from the BS plates give atypical reactions on TSI and LIA, are at least 2 additional typical colonies picked, if available? Yes No N/A

j. Are selective agar plates stored at 5 – 8°C until completion of confirmation steps? Yes No N/A

14. If suspicious colonies are not well isolated, are they re-streaked for purification directly onto selective agar plates before inoculating Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants? Yes No N/A

15. Are characteristic colonies inoculated to TSI slants and LIA slants by inoculating the slants in tandem with a single pick from a colony, and by stabbing the butts and streaking the slants in one operation? Yes No N/A

16. After incubation at 35 ± 2°C for 24 ± 2 hours with caps loosened, are TSI and LIA slants with the following

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

characteristics of *Salmonella* selected for further analysis:

a. **LIA** – Alkaline slant and butt (purple throughout) with or without hydrogen sulfide (H<sub>2</sub>S) production? Yes No  
 N/A  
 (Note: Some strains will produce an acid butt, along with a typical TSI slant.)

b. **TSI** – Alkaline (red) slant and acid (yellow) butt with or without hydrogen sulfide (H<sub>2</sub>S) production? Yes No N/A  
 (Note: Some strains will produce an acid slant and butt.)

17. Are TSI/LIA cultures, which appear to be mixed, streaked for isolation before additional biochemical or serological tests are performed? Yes No N/A

18. Before reporting a presumptive positive sample as negative, at least six TSI/LIA cultures (if available) picked as below are subjected to further biochemical and serological testing:

a. For FSIS MLG 4 method: are at least three well isolated colonies from each of two plating media picked to TSI/LIA pairs and subject to confirmation testing before a sample is reported as negative? Yes No N/A

b. For AMS or FDA method, are at least two well isolated colonies from each of three plating media picked to TSI/LIA pairs and subject to confirmation testing before a sample is reported as negative? Yes No N/A

19. Is a rapid/miniaturized biochemical test system used for identifying *Salmonella*? Yes No N/A

If yes, list the test system below with its AOAC reference number:

Biochemical Test System: \_\_\_\_\_

AOAC Reference Number: \_\_\_\_\_

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

- |     |  |     |     |     |
|-----|--|-----|-----|-----|
| 20. | Are the manufacturers' guidelines for miniaturized biochemical systems followed for inoculum preparation, incubation, and interpretation of results?   | Yes | No  | N/A |
| 21. | Are sufficient biochemical tests performed to presumptively identify atypical isolates as <i>Salmonella</i> ? (i.e. urease, dulcitol, lactose, and sucrose fermentation, and malonate utilization) | Yes | No  | N/A |
| 22. | When performing slide agglutination tests, are all materials and equipment brought to room temperature before testing?   | Yes | No  | N/A |
| 23. | Is a saline control included to detect autoagglutination when performing the polyvalent or group somatic (O) antigen slide agglutination test?   | Yes | No. | N/A |
| 24. | Are polyvalent flagellar (H) antigen screening tests performed by a tube method using formalinized cultures prepared from:   |     |     |     |
|     | a. Brain-heart infusion broth incubated at 35°C for 4 to 6 hours for same-day testing?   | Yes | No  | N/A |
|     | b. Trypticase soy broth incubated 24 hours at 35°C for next-day testing?   | Yes | No  | N/A |
| 25. | For H antigen testing is a negative control of formalinized saline with the formalinized culture included in the testing?  | Yes | No  | N/A |
| 26. | Are H antigen tests incubated at 48 – 50°C for 1 hour?   | Yes | No  | N/A |
| 27. | Are diluted <i>Salmonella</i> H antisera prepared in quantities sufficient only for daily use, and any remaining diluted antisera discarded at the end of the day?                                 | Yes | No  | N/A |
| 28  | If the Oxoid kit or SSI H antiserum is used, are manufacturer's instructions followed?   | Yes | No  | N/A |

U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

*Safety issues are not within the scope of the PEPRLab Program audit. Therefore, the laboratory is not required to report a corrective action for any observations and/or recommendations resulting from this part of the review. This segment is conducted out of concern for the health and safety of laboratory personnel.*

**H. SAFETY:**

Facilities need to be designed and equipped to meet established OSHA safety standards. Protective equipment should be available to personnel and a comprehensive safety program should be included in laboratory procedures.

- |    |  |   |     |    |     |
|----|--|---|-----|----|-----|
| 1. | Is there an ongoing, documented safety education program that includes, but is not limited to, instruction on: |   |     |    |     |
|    | a.   | Location and use of fire extinguishers, blankets, and other safety equipment? | Yes | No | N/A |
|    | b.   | Fire drills and evacuation routes?  | Yes | No | N/A |
|    | c.   | Handling emergency situations?  | Yes | No | N/A |
|    | d.   | Basic first aid procedures?   | Yes | No | N/A |
|    | e.   | CPR training?   | Yes | No | N/A |
|    | f.   | The labeling of all cancer suspect agents?                                    | Yes | No | N/A |
|    | g.   | Lifting heavy items?  | Yes | No | N/A |
|    | h.   | "Right to Know" laws?   | Yes | No | N/A |
| 2. | Is there a safety manual available in the laboratory?  |   | Yes | No | N/A |
| 3. | Does it include procedures for:  |   |     |    |     |
|    | a.   | Handling spills of contaminated materials?                                    | Yes | No | N/A |
|    | b.   | Disposal of biological waste?   | Yes | No | N/A |

U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

c. Disposal of chemical waste?	Yes	No	N/A
d. Handling toxic materials?	Yes	No	N/A
4. Are Materials Safety Data Sheets (MSDS) available in the laboratory for all chemicals used in the laboratory?	Yes	No	N/A
5. Is there a designated safety officer in the laboratory?	Yes	No	N/A
6. Does the safety officer conduct periodic safety inspections using a checklist?	Yes	No	N/A
7. Are safety deficiencies and corrective actions documented?	Yes	No	N/A
8. Are accidents documented and reported to the safety officer?	Yes	No	N/A
9. Is emergency medical help readily available if needed by laboratory personnel?	Yes	No	N/A
10. Are emergency phone numbers (i.e. fire, ambulance, police) posted in a conspicuous place on or near the phone?	Yes	No	N/A
11. Are personnel ever alone in the laboratory?	Yes	No	N/A
12. Does the laboratory have at least two exits and are all exits and hallways free of obstructions?	Yes	No	N/A
13. Can the doors be locked from both sides?	Yes	No	N/A
14. Are the following in the laboratory:			
a. Fire extinguishers (CO <sub>2</sub> , dry chemical)?	Yes	No	N/A
b. Fire blanket?	Yes	No	N/A
c. Eyewash station?	Yes	No	N/A
d. Overhead shower?	Yes	No	N/A



U.S. DEPARTMENT OF AGRICULTURE  
 FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
 Program**

- |     |  |     |    |     |
|-----|--|-----|----|-----|
| e.  | Fire alarm system?   | Yes | No | N/A |
| f.  | Sprinkler system?  | Yes | No | N/A |
| g.  | First aid kit?   | Yes | No | N/A |
| 15. | Are fire extinguishers and other safety equipment regularly inspected, certified to be in working order, and their condition documented?   | Yes | No | N/A |
| 16. | Is an EPA-approved disinfectant available to clean up biohazardous spills and disinfect bench tops daily?  | Yes | No | N/A |
| 17. | Is the disinfectant prepared and used according to the manufacturers instructions?   | Yes | No | N/A |
| 18. | Are biohazardous materials discarded in leak-proof, tear-resistant plastic bags marked with a biohazard symbol?  | Yes | No | N/A |
| 19. | Are biohazardous waste materials steam sterilized at 121°C for at least 45 minutes, with biohazard bags vented to effect complete sterilization as required by the manufacturer, or else incinerated prior to disposal in landfills?   | Yes | No | N/A |
| 20. | Are biohazardous materials removed from the laboratory daily and contained in a manner to minimize accidental spills during storage and transport and to exclude rodents and vermin? (i.e. Bags are tied and placed in covered, rigid containers such as buckets, cans, or cardboard boxes, and liquids are placed in capped or tightly stoppered bottles or tubes.) | Yes | No | N/A |
| 21. | Are janitors and other maintenance personnel instructed in proper methods of disposal, and are disposal areas located well away from the building and protected from trespassers?  | Yes | No | N/A |
| 22. | Are personnel instructed not to taste chemicals at all, and not to directly smell chemicals?   | Yes | No | N/A |

U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

- |     |   |     |    |     |
|-----|---|-----|----|-----|
| 23. | Is mouth pipetting strictly prohibited (with no exceptions for sterile solutions)?  | Yes | No | N/A |
| 24. | Are eating, drinking, and smoking prohibited in the laboratory, and is labware prohibited from use for any of these purposes?                   | Yes | No | N/A |
| 25. | Is food prohibited from refrigerators that are used for reagents, samples, etc.?  | Yes | No | N/A |
| 26. | Are personnel instructed to wash hands after handling samples, working with cultures, handling chemicals, and/or before leaving the laboratory? | Yes | No | N/A |
| 27. | Are laboratory personnel required to confine long hair?   | Yes | No | N/A |
| 28. | Are aprons, gloves, and goggles available for handling hazardous materials?   | Yes | No | N/A |
| 29. | Are heat-resistant gloves available near the autoclave and in the media preparation area?   | Yes | No | N/A |
| 30. | Are laboratory coats or other protective clothing worn only in the laboratory?  | Yes | No | N/A |
| 31. | Are bunsen burners turned off when not in use?  | Yes | No | N/A |
| 32. | Are chipped, broken, or etched glassware discarded in a specially marked, puncture proof, sealed container?                                     | Yes | No | N/A |
| 33. | Is broken glassware always cleaned up with a dust pan/brush and never picked up with the hands?   | Yes | No | N/A |
| 34. | Are heavy plastic carriers available for transporting acids or other corrosive chemicals?   | Yes | No | N/A |
| 35. | Are bottles of acid (HC1) always tightly capped and rinsed on the outside after being used and/or before  |     |    |     |

U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

	being opened?	Yes	No	N/A
36.	Have laboratory personnel been taught to always pour acid into water, never water into acid?	Yes	No	N/A
37.	Is a safety cabinet or room available for storing large containers of hazardous chemicals?	Yes	No	N/A
38.	Are electrical connections covered with a heavy rubber coating?	Yes	No	N/A
39.	Are extension cords grounded and, if running across the floor, are they taped down?	Yes	No	N/A
40.	Are all electrical cords, receptacles, and switches in good condition and located away from water sources?	Yes	No	N/A
41.	Does the laboratory use mercury thermometer(s)? If so, is a mercury spill kit available?	Yes	No	N/A
42.	If located near water sources, are electrical outlets protected with ground-fault circuit interrupters?	Yes	No	N/A

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

**I. SUMMATION AND COMMENTS:**

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

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2. Handbook of Microbiological Media, 3<sup>rd</sup> Edition, 2004, CRC Press, Boca Raton, FL.
3. Biosafety in Microbiological and Biomedical Laboratories, 4<sup>th</sup> ed. May 1999. Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, GA.
4. Compendium of Methods for the Microbiological Examination of Foods, 4<sup>th</sup> ed. 2001. APHA, Technical Committee on Microbiological Methods for Foods, Washington, DC.
5. Good Laboratory Practice Regulations, Code of Federal Regulations (CFR), 21 CFR Part 58, U.S. Food and Drug Administration, 5600 Fishers Lane, Rockville, MD.
6. Difco & BBL Manual, 1st ed., 2003, Becton, Dickson and Company, Sparks, Maryland.
7. Official Methods of Analysis of AOAC INTERNATIONAL, Current AOAC Internet Version
8. Laboratory Methods for Egg Products – Section I (1993 revision) and Section VII (1994 revision), U. S. Department of Agriculture, Agriculture Marketing Service, Washington, D. C.
9. Microbiology Laboratory Guidebook online (MLG), Chapter 4, U. S. Department. of Agriculture, Food Safety and Inspection Service, Washington, D.C.
10. Bacteriological Analytical Manual online (BAM), Chapter 5, U.S. Food and Drug Administration, Washington, D.C.

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U.S. DEPARTMENT OF AGRICULTURE  
FOOD SAFETY AND INSPECTION SERVICE

**Pasteurized Egg Products Recognized Laboratory (PEPRLab)  
Program**

**Instructions for completing the form**

1. Answer all questions on the checklist by placing a circle around the appropriate response or by filling in the blank. Responses are based on observations or information supplied by laboratory personnel. Questions pertaining to services, equipment, instruments, methods or procedures not used routinely by the laboratory should be marked as not applicable (N/A).
2. Scan and submit the completed form to: [PEPRLab@fsis.usda.gov](mailto:PEPRLab@fsis.usda.gov)
3. Alternatively, mail the completed form to:

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