

## Detection of Listeria by GDS

TP-M: 007

Rev: 01

Date: 9/5/07

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**Supersedes:** Rev 00  
**Change:** Add instructions for tapping of Pick Pen tips and mixing of Polymerase. Add information on holding times at various steps.  
**Reason:** Testing guidelines emphasized by Biocontrol representative.

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**SCOPE:**

This test method is for the specific detection of Listeria species or mono in food and non-food items by the GDS.

**EQUIPMENT:**

Sterile plastic (stomacher) bags  
Analytical balance, accurate to 0.1g minimum  
Incubator, capable of maintaining 35°C +/- 2°C  
Sterile pipets  
Sample holder  
Stomacher mixer (optional)  
GDS analyzer

**REAGENTS:**

Prepared Demi Fraser Broth  
GDS Listeria Test Kit (Species or Mono, depending on requirement)

**PREPARATION:**

1. Open a sterile bag and place it in a sample bag holder on the balance. Tare the balance.
2. Weight the appropriate amount of sample into a sterile bag. Typically, 25 grams of sample is used for the test. For some samples, a larger amount may be required. Record the weight used in the log book if other than 25 grams.
3. For food samples, mix the sample as homogenous as possible. If the sample consists of a variety of materials that cannot be homogenized, take representative portions of the various materials for the test.
4. Mark the plastic bags with the sample number, the date and test name.

**PROCEDURE:**

1. Add enough Demi-Fraser broth to the sample in the bag to create a 1:10 dilution (i.e. for 25 grams, add enough broth to bring the final weight to 250 grams). Squeeze and shake the item in the solution to thoroughly mix. Close the bag or container and place it upright in a sample holder to be placed in the incubator.
2. Place the prepared samples in the incubator at 30°C for 30 - 48 hours.
3. After incubation, remove the sample from the incubator.
4. Remove the Listeria Test Kit from the refrigerator. (Use the appropriate kit for the type of Listeria testing required.) Shake the concentration reagent to mix well. Pipet 20µl of concentration reagent into the wells of a plastic GDS sample block, filling one well for each sample to be tested, in one vertical column of the sample block. Cover the column with an adhesive strip after pipetting.
5. Pipet 1ml of wash solution into each well in the next column of the sample block, filling one well for each sample to be tested. Cover the column with an adhesive strip after pipetting.
6. Pipet 35µl of resuspension buffer into the wells of a GDS resuspension block, filling one well for each sample to be tested, in one vertical column of the sample block. Cover the column with an adhesive strip after pipetting. (Note: solutions in the sample block and resuspension plate may sit for a maximum of 1 hour at room temperature before proceeding to step 7, or up to 4 hours if refrigerated.)
7. Pipet 1 ml of the incubated sample solution into a well containing the 20µl of concentration reagent. Record the sample number being placed in each well on a separate piece of paper.
8. Recover the wells with the adhesive strip and place the sample block on the vortex shaker to mix. Mix for 10 – 15 minutes.
9. After mixing, remove the sample block from the vortex shaker. Place disposable tips on the PickPen and extend the PickPen tips by pressing the orange lever. Place the PickPen tips into the sample cells. Mix the Pick Pen tips back and forth in the solution for 30 seconds. Lift the tips out of the solution and tap them against the sides of the block to remove excess solution. Then transfer the PickPen into the next column of the sample block containing the wash solution.

**Written by:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Approved by:** \_\_\_\_\_ **Date:** \_\_\_\_\_

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10. Mix the Pick Pen tips back and forth in the wash solution of 10 seconds. Lift the tips out of the solution and tap them against the sides of the block to remove excess solution.
11. Place the tips into the buffer solution in the resuspension plate. Release the orange lever to pull back the Pick Pen tips. Mash the Pick Pen tip covers in the resuspension solution to transfer the sample material into the resuspension solution. Recover the resuspension wells with the adhesive strip. The samples may sit for up to one hour at room temperature or 8 hours with refrigeration before proceeding to Step 12.
12. Place the resuspension plate into the incubator at 60°C for 15 – 30 minutes.
13. During the last few minutes of the incubation period, prepare the sample ampules. Place the ampules needed, one per sample, into a metal cooling block that has been refrigerated for a minimum of 20 minutes. Add 10µls of Polymerase solution to each small plastic ampule. NOTE: Always mix the Polymerase solution well before each use by moderate shaking. Failure to mix the Polymerase solution before each use can cause inadequate test response for the internal control.
14. Remove the resuspension plate from the incubator and use the pipet to transfer 20µls of sample from the resuspension block into the ampule tubes. Close the tubes and flip them to mix.
15. Place the tubes in the GDS sample rack. The tubes must be placed in the GDS machine within 15 minutes after addition of the sample. Attach the locking ring.
16. Enter the sample numbers into the computer. (This step can be performed while samples are shaking or incubating also.) Open a New Run on the computer screen. Enter the sample numbers in the sample list in order by which they will be tested. Enter the GDS kit lot number for each sample and select the test to be performed on each sample. Save the file.
17. Press “Start Run” on the computer screen after the sample names are all entered and the sample vials are loaded into the sample rack.

**RESULTS/EVALUATION:**

1. The GDS system will report sample results as positive or negative for Listeria mono. Positive results are shown in red and appear on the graph on the screen as a line that crosses the red testing threshold line. Record the results as listed by the GDS when the run is completed.
2. Insure that the internal control lines on the GDS run have crossed the threshold. If an internal control line is not correct for any sample, the sample must be rerun or tested by another method.
3. In case of positive results, the result should be confirmed by test method TP-M 008.

**REFERENCES:****AOAC 070701 & AOAC 070702**