

Detection of E. Coli 0157 by GDS

TP-M: 010

Rev: 06

Date: 8/1/10

Supersedes: Rev 05
Change: Change dilution volume for large meat samples to 1:5
Reason: Reduced volume of media needed per approved method validation study

SCOPE:

This test method is for the specific detection of E. coli 0157 in food and non-food items by the GDS. This method meets or exceeds the sensitivity of USDA method MLG Chapter 5.

EQUIPMENT:

Sterile plastic (stomacher) bags
Analytical balance, accurate to 0.1g minimum
Incubator, capable of maintaining 42°C +/- 2°C
Sterile pipets
Sample holder
Stomacher mixer (optional)
GDS analyzer

REAGENTS:

Prepared mEHEC Broth
GDS E. coli Test Kit

PREPARATION:

1. Open a sterile bag and place it in a sample bag holder on the balance. Tare the balance.
2. When preparing meat samples of more than 25 grams, preheat the mEHEC broth in the 42°C incubator for a minimum of 2 hours before use.
3. Weigh the appropriate amount of sample into a sterile bag. For all raw meat products, use 375 grams of sample. For all other types of products, use 25 grams. If not enough sample is provided to meet these weights, use as much sample as has been provided. Record the actual weight used in the log book.
4. 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.
5. Mark the plastic bags with the sample number, the date and test name.

PROCEDURE:

1. Add enough sterile preheated mEHEC broth to the sample to create a 1:10 dilution. For meat samples of over 100 grams, use a 1:5 dilution volume. (Example: 375g sample plus 1500g of media to final weight of 1875g). Squeeze and shake the item in the solution to thoroughly mix. A stomacher mixer may also be used to thoroughly mix the sample. Close the bag or container and place it upright in a sample holder to be placed in the incubator.
2. For swab samples, add enough sterile mEHEC broth to the container or bag containing the swab so that it is thoroughly immersed in the broth. Squeeze sponge-type swabs in the broth to saturate the swab with broth.
3. Place the prepared samples in the incubator at 42°C.
4. The recommended incubation period for this test is 6.5 – 18 hours. The routine incubation time for all E. coli 0157:H7 samples will be approximately 15 – 18 hours, starting the incubation on the afternoon of the date received and concluding the following morning. The minimum incubation time for this test is 6.5 hours for swabs and samples of 200g or less, and 8 hours for samples greater than 200g. The minimum incubation times shall only be used in the event of an emergency/rush request from the customer. The maximum incubation period of 18 hours should not be exceeded, as the mEHEC broth is designed for rapid growth of the organism, and incubation periods beyond 18 hours can lead to die off of the E. coli 0157:H7 organisms.
5. After incubation, remove the sample from the incubator.
6. Remove the E. coli Test Kit from the refrigerator. Shake the concentration reagent to mix well. Pipet 20µls 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.
7. 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.

Written by: _____ **Date:** _____

Approved by: _____ **Date:** _____

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8. Pipet 35µls 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.)
 9. Pipet 1 ml of the incubated sample solution into a well containing the 20µls of concentration reagent. Record the sample number being placed in each well on a separate piece of paper.
 10. Recover the wells with the adhesive strip and place the sample block on the vortex shaker to mix. Mix for 5 – 15 minutes.
 11. 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.
 12. 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.
 13. 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 13.
 14. During the mixing period or within the recommend hold time, prepare the sample ampule tubes. 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.
 15. Use the pipet to transfer 20µls of sample from the resuspension block into the ampule tubes. Close the tubes and flip them to mix.
 16. 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.
 17. Enter the sample numbers into the computer. (This step can be performed while samples are mixing 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.
 18. 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 E. coli 0157. 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. Record the result as Positive or Negative per the amount of sample used. For raw meat samples, if less than 375g have been provided for the test, record on the report that the entire sample was used to for testing.
3. 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.
4. This method meets or exceeds the sensitivity of the USDA method. In case of a positive result, the sample should be confirmed by test method TP-M002 (based on USDA Method MLG Chapter 5, Sections 5.4 & 5.5).

REFERENCES:

AOAC 2005.04