Certification Report

AOAC Research Institute

Performance Tested Method
030201

GENE-TRAK®
Salmonella Microwell assay

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The GENE-TRAK *Salmonella* Microwell assay has been validated and certified as a *Performance Tested Method*™ by the AOAC Research Institute as an effective method for the detection of *Salmonella* spp. in a wide variety of foods, with low rates of false negative and false positive reactions.

The GENE-TRAK® *Salmonella* Microwell assay is a DNA probe based diagnostic in kit format which permits rapid and accurate detection of *Salmonella* spp. in foods. Following sample pre-enrichment, selective enrichment, and post enrichment incubation periods, target bacteria are lysed enzymatically at 65°C and *Salmonella* -specific oligonucleotide probes are added for a 60 minute hybridization incubation at 45°C. If *Salmonella* ribosomal RNA (rRNA) is present in the test sample, detector probe directly labeled with horseradish peroxidase (HRP) and polydeoxyadenylic acid (poly dA)-tailed capture probe hybridize to the target organism rRNA sequences. Concurrently, base pairing between the poly dA tailed capture probe and polydeoxythymidylic acid (poly dT) coated polystyrene microwells facilitates solid phase capture of the probe-target hybrid molecules. Unbound probe is removed by washing, and substrate chromogen is added to each well. The reaction of HRP with substrate chromogen produces a blue color. The reaction is stopped with the addition of sulfuric acid which changes the color of the substrate from blue to yellow. A microwell plate or microwell strip reader (A_{450}) measures absorbance: absorbance in excess of the threshold value indicates the presence of *Salmonella* spp. in the test sample. Positive assay results must be confirmed by standard culture methods.

1. Target Organism

Serovars of *Salmonella enterica*. The DNA probes used in the method are not reactive with *Salmonella bongori.*

2. Matrices

All foods.

3. Summary of validated performance claims

Sensitivity	$\geq$ 99%
Specificity	$\geq$ 97%
Agreement with BAM/AOAC culture method	$\geq$ 99%

4. Accuracy/ Comparison to Existing Methods

A total of 1250 food samples were tested for *Salmonella* spp., of which 880 were confirmed positive by traditional serological and biochemical procedures for the presence of *Salmonella* spp. The manual microwell method detected 873 of 880 total positives for a sensitivity of 99.2%. There were 11 unconfirmed positive assay results by the manual microwell method resulting in a specificity of 97.0%. The automated microwell method detected 871 of 880 total positives for a sensitivity of 99.0% and a specificity of 93.8% was recorded (23 unconfirmed positive assay results). The dipstick-format method detected 873 of the 880 total positives for a sensitivity of 99.2% and the specificity was 94.6% (20 unconfirmed positive assay results). The BAM/AOAC method detected 875 of the total positives resulting in a sensitivity of 99.4%.

Agreement between the methods was as follow:

Manual microwell vs. Automated microwell	99.6%
Manual microwell vs. Dipstick	99.8
Manual microwell vs. BAM/AOAC culture	99.0
Automated microwell vs. dipstick	99.8
Automated microwell vs. BAM/AOAC culture	98.8

Results of the 20-food trial clearly show that the microwell assay, whether performed manually or by an automated instrument, is capable of detecting low levels of *Salmonella* spp. in a wide variety of foods.

False negative rates of 1% or less were observed, and further the microwell method showed very close agreement (98.8% or greater) to both the BAM/AOAC culture method and the established GENE-TRAK *Salmonella* DLP dipstick probe assay. The assay has also been proven to be very specific, with no cross reactions observed in exclusivity testing and a false positive rate of less than 3% exhibited by the manual microwell assay in testing of foods. A somewhat higher rate of unconfirmed positive reactions was observed with the automated version of the assay compared to the manual version. In both cases, there was no apparent pattern to these false positive results; they did not appear to be related to the food matrix or any inherent reactivity of the probes with particular competitor organisms.

The later statement is supported by the results of exclusivity testing and also by the fact that, in all but one case (chocolate), false positive reactions occurred by only one or two of the three probe methods in any given experiment. This is relevant since the probe set used is the same for the three probe tests. It is thought that these false positive results are random; possible causes include incomplete washing of individual wells and rare splashing of sample or reagents from well to well during pipetting. This latter procedural difficulty is inherent in the design of microwell based assays in which the coated well reaction vessels are in extremely close physical proximity. This situation will be exacerbated in cases where the majority of samples tested contain the target organism, such as the case here. In routine quality control testing in which the majority of samples are negative, we expect false positive rates to be lower.

Results of the external validation study support the conclusion that the microwell assay displays a sensitivity comparable to that of the BAM/AOAC culture method when used for the detection of *Salmonella* spp. in a variety of foods. The microwell and BAM/AOAC methods produced a comparable number of positive results for each of the three food types tested as determined by χ^2 analysis. Only one apparent false positive result was obtained.

5.0 Test Kit Information

5.1 Kit name - GENE-TRAK® *Salmonella* Microwell Assay

5.2 Catalog number – 6700

5.3 Ordering information

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5.4 Test kit reagents

5.4.1 *Microwell plate, 96 coated wells in divisible strips*. Polystyrene microwells, high binding, 12 rows by 8 well configuration, coated with polydeoxythymidylic acid (poly dT).

5.4.2 *Lysis Reagent Concentrate (Reagent 1A, 2 vials).* Contains 25 mg lyophilized Proteinase K. Once Reagent 1A has been reconstituted, it must be stored at -20°C and is stable for 60 days.

5.4.3 *Lysis Reagent Buffer (Reagent 1B, 12 mL).* Contains 0.26 M Tris pH 7.4, 5 mM disodium EDTA, 5% n-lauryl sarcosine, and 0.01% bromphenol blue.

5.4.4 *Hybridization Solution (Solution 2, 18 mL)*. Contains formamide (*Caution:* Avoid contact with skin; if contact occurs, wash skin thoroughly with water).

5.4.5 *Salmonella Probe Solution (Solution 3, 6 mL).* Contains *Salmonella*-specific oligonucleotide labeled with horseradish peroxidase (detector probe) and *Salmonella*-specific oligonucleotide 3' labeled with polydeoxyadenylic acid (capture probe) in 4.8 M Tris pH 7.0, 1 mM sodium hydroxide, and 5% cresol red.

5.4.6 *Wash Solution 20X Concentrate (Solution 4, 25 mL)*. Contains 1.0 M Tris, pH 7.5, 0.04 M EDTA, 3.0 M NaCl, and 2% Tween-20.

5.4.7 *Substrate Chromogen Solution (Solution 5, 15 mL).* Standard horseradish peroxidase detection reagent for use in ELISA and other HRP-based colorimetric assays. Contains urea peroxide and tetramethlybenzidine.

5.4.8 *Stop Solution (Solution 6, 5 mL).* Contains 4.0 N sulfuric acid. (*Caution*: Corrosive. Avoid contact with skin; if contact occurs, wash skin thoroughly with cold water).

5.4.9 *Positive Control (5 mL).* Contains formaldehyde-inactivated *Salmonella*

Typhimurium in concentrations sufficient to produce absorbance > 1.0 when tested in assay. Actual cell concentration may vary depending on strain or organism employed, media, and conditions used for its preparation.

5.4.10 *Negative Control (5 mL).* Contains formaldehyde-inactivated *Citrobacter freundii* in concentrations sufficient to produce absorbance > 0.15 in assay when stringency conditions of assay are incorrect (e.g., low hybridization temperature). Actual cell concentration used may vary depending on strain of organism employed, media, and conditions used for its preparation.

Items 5.4.1 – 5.4.10 are available as GENE-TRAK® *Salmonella* Microwell assay from Neogen Corp.. Store microwell plate, Positive Control, Negative Control, and Reagents 1A, 2, 3 and 5 at 2-8°C. Other reagents may be stored at 2-25°C. Alternatively, the entire kit may be stored at 2-8°C.

6.0 Additional supplies and reagents

6.1 *Preenrichment medium.* Refer to [1]. Available from Acumedia (Baltimore, MD) and other vendors.

6.2 *Selective enrichment media.* Refer to [2]. (a) Tetrathionate broth, (b) Rappaport Vassiliadis broth. Available from Acumedia (Baltimore, MD) and other vendors.

6.3 *Gram Negative (GN) broth.* Refer to [3]. Available from Acumedia (Baltimore, MD) and other vendors.

6.4 *Diagnostic reagents.* As necessary for culture confirmation of positive DNA hybridization assays [1].

6.5 Blender or homogenizer.

6.6 Culture bottles for sample pre-enrichment.

6.7 Culture bottles for 11 mL sample volume.

6.8 Test tubes, glass, 12 x 75 mm.

7. Apparatus

7.1 Blender or homogenizer.

7.2 Micropipette and tips to dispense 145 µL volume.

7.3 Repeater pipette or 8-channel pipette and tips to dispense 50 µL, 100 µL, 125 µL, and 150 µL volumes.

7.4 Incubator at 35°C +/- 1°C.

7.5 Water bath or heater block at 42°C +/- 0.2°C.

7.6 Water bath or heater block at 65 +/- 1 ^{o}C.

7.7 Small orbital platform shaker capable of 150 rpm.

7.8 Air incubator at 45°C +/- 1°C.

7.9 Vacuum source with trap (required for some types of strip washers).

7.10 Microwell plate or strip washing device (e.g., strip washer with 8 well orientation).

7.11 Microwell plate or strip reader at 450 nm with discrimination of 0.01 absorbance unit

7.12 Automated instrument capable of performing assay as described, e.g., Personal Lab®, BioChem ImmunoSystems, Allentown, PA (not required if performing assay manually).

References

1. U.S. Food and Drug Administration. 1998. *Bacteriological Analytical Manual*, 8th ed., Rev. A, AOAC International, Gaithersburg, MD, Chapter 5.

2. U.S. Food and Drug Administration. 1998. *Bacteriological Analytical Manual*, 8th ed., Rev. A, AOAC International, Gaithersburg, MD, Appendix 3.

3. Atlas, R.M. 1997. *Handbook of Microbiological Media*, 2nd ed., CRC Press, Boca Raton, FL, pg. 606.