GENERAL DESCRIPTION
Assurance Gold Salmonella EIA is an enzyme immunoassay that detects all motile and non-motile serotypes of Salmonella. It has been specifically formulated to minimize cross-reactivity with many Enterobacteriaceae while maintaining superior sensitivity. Results of the Assurance Gold EIA are designed to be read visually using the color standard provided or instrumentally with a microplate reader.

USING Assurance Gold Salmonella EIA

A. Sample Preparation and Enrichment

(a) Pre-Enrichment

Processed Foods: Add 25 g of sample to 225 mL of appropriate pre-enrichment broth, prewarmed to 35-37°C, as in Appendix I & II. Incubate 6-8 h at 35-37°C.

Dried Powder Processed Foods: Add 25 g of sample to prewarmed 225 mL of Brain Heart Infusion broth + 1 mL enrichment supplement containing Oxylase™ (BHI+0) as in Appendix III. Incubate 6-8 h at 35-37°C.

Raw Foods: Add 25 g of sample to 225 mL of Buffered Peptone Water + novobiocin as in Appendix IV. Incubate 18-24 h at 35-37°C.

(b) Selective Enrichment

Processed Foods: Transfer 25 mL of pre-enrichment broth to 5 mL of a 5X concentrated Rappaport-Vassiliadis (5XRV) broth and another 25 mL to 5 mL of a 5X concentrated Tetradionate (5XTT) broth. Incubate 16-24 h in 42°C waterbath.

Dried Powder Processed Foods: Add 32 mL BHI 0 to 5 mL 5X TT. Incubate 16-24 h in 42°C waterbath.

Raw Foods: Transfer 0.1 mL pre-enrichment broth to tube containing 10 mL RV broth and another 1.0 mL to tube containing 10 mL of TTB broth. Incubate 5-8 h at 42°C water bath.

(c) Post-Enrichment

Processed Foods: Following selective enrichment incubation, transfer and combine 1.0 mL of TT and 0.5 mL of RV broths into a single tube filled with 10 mL prewarmed Trypsinase Soy Broth + novobiocin (TSB+nv). Label tubes. Incubate 6-8 h at 35-37°C.

Dried Powder Processed Foods: Transfer 1.0 mL of TT into a single tube of 10 mL prewarmed TSB+nv. For dried egg products, transfer 0.2 mL of TT broth into a single tube of 10 mL prewarmed TSB+nv. Label tubes. Incubate 6-8 h at 35-37°C.

Raw Foods: Following selective enrichment incubation, transfer and combine 1.0 mL of TT and 0.5 mL of RV broths into a single tube filled with 10 mL prewarmed TSB+nv. Label tubes. Incubate 16-20 h at 42°C water bath.

Following TSB+nv incubation, vortex mix tube contents and transfer 1.0 mL to a test tube.

NOTE: Retain original TSB+nv broth under refrigeration (2-8°C). Use for alternate Washing Procedure (see section E).

B. Reagent Preparation

Before beginning the assay, prepare reagents and allow all kit components to reach ROOM TEMPERATURE. Verify that extraction reagent is homogenous. Store unused microwells in the sealed foil container. This volume is sufficient to wash each well at 15 min. The blue color will turn yellow. After adding stop solution, read and record results.

NOTE: To get valid results, the microwell plate reader must be calibrated against the BLANK well before reading samples and Control. (1) Standardize reader by reading the BLANK well and adjusting optical density (O.D.) to zero. (2) Read sample absorbancies of each well, starting with the two Positive Controls. When reader is standardized to BLANK well, certain samples may read less than zero O.D. (a negative reading). This is not uncommon and indicates a negative result.

NOTE: Microwell plate reader linear range is variable depending upon manufacturing specifications. If PC is reported as “over” or numerical value that exceeds 2.5, use 2.5 for calculation purposes.

C. Test Procedure

(a) Fit required number of microwells into holder. Reseal unused microwells in foil pouch. In addition to samples, allow three extra wells for 2 Positive Controls (PC) and 1 Blank. Carefully record Positive Controls, Blank and sample positions in holder.

(b) Vortex mix samples and Positive Control before pipetting. A new pipet tip must be used for each sample. Pipette 100 μL of sample into each prewarmed 100 μL of Reagent 3 - Positive Control into each Positive Control well. LEAVE BLANK WELL EMPTY. Cover and incubate 30 min at 35-37°C. Do not stack anything on top of microwell holder during incubation. Do not agitate plate during any incubation step.

(f) Following incubation, wash each well three times according to the following procedure:

Washing Procedure: Completely remove contents of well with a microwell washer. Immediately following aspirations, fill wells with Wash Solution.

Alternate Washing Procedure: Is acceptable to:

(1) Remove contents of well by inverting and vigorously tapping plate; (2) Wash wells by filling each well with wash solution using a wash bottle. Repeat twice for a total of three aspiration/wash cycles per step. Avoid overfilling wells to prevent antigen carry-over to adjacent non-reagent wells. Avoid underfilling wells to prevent ineffective washing. Effective washing is critical to obtaining accurate data. Remove excess wash solution by inverting wells and tapping prior to proceeding to next step.

(d) Immediately following removal of the third wash, add 100 μL Reagent 4 - Conjugate to each well, including the Positive Control and Blank wells. Cover and incubate 30 min at 35-37°C.

(e) Following incubation, aspirate and wash each well three times. Refer to C(c) for Washing Procedure.

(f) Immediately following removal of the third wash, add 100 μL of Reagent 5 - Substrate to each well, including Positive Control and Blank wells. Incubate at room temperature for 10-15 min. DO NOT WASH WELLS. Proceed immediately to D Reading Results.

D. Reading Results

Results may now be read visually OR instrumentally.

(a) Visual Reading: Using the Color Standard

Since color development will continue, reading must be made within allotted 10-15 min. after adding substrate. Place well holder onto a white background. Looking straight down into wells, compare color at center of well to color standard card. The edges of the wells may reflect the color of adjacent wells and appear darker, this should be disregarded. Sample wells that are at least as dark as Color II (the Positive Control) are presumptive positive and should be culturally confirmed (See Section E).

(b) Instrumental Results: Using a Plate Reader

Fit microwell reader with 450 nm filter. Add 100 μL of Reagent 6 - Solution to each well at 15 min. The blue color will turn yellow. After adding stop solution, read and record results.

NOTE: To get valid results, the microwell plate reader must be calibrated against the BLANK well before reading samples and Control. (1) Standardize reader by reading the BLANK well and adjusting optical density (O.D.) to zero. (2) Read sample absorbancies of each well, starting with the two Positive Controls. When reader is standardized to BLANK well, certain samples may read less than zero O.D. (a negative reading). This is not uncommon and indicates a negative result.

NOTE: Microwell plate reader linear range is variable depending upon manufacturing specifications. If PC is reported as “over” or numerical value that exceeds 2.5, use 2.5 for calculation purposes.

E. Interpretation of Test Results

Control Value

The Positive Control absorbance values should be greater than or equal to 0.8 O.D. units. Absorbance values that fall below this value may indicate problems with Washing Procedure.

Cutoff Value

Calculate the average value of the two Positive Control readings and multiply by 0.25 to establish the cutoff value:

PC1 + PC2 X 0.25 = Cutoff Value

where PC1 & PC2 = Positive Control absorbance values (O.D. units). Include Positive Controls in each test run.

Positive Results

Samples with absorbance values greater than or equal to the Cutoff Value are presumptively positive. Positive samples should be confirmed using culture methods described in BAM/AOAC or USDA methodology. Streak from refrigerated retained broth.

Negative Results

Samples with absorbance values less than the Cutoff Value are negative.

COMPONENTS

Each Assurance Gold Salmonella EIA kit contains the following:

TEST WELLS

REAGENT 1 - EXTRACTION REAGENT

REAGENT 2 - WASH SOLUTION CONCENTRATE

REAGENT 3 - POSITIVE CONTROL

REAGENT 4 - CONJUGATE

REAGENT 5 - SUBSTRATE

REAGENT 6 - STOP SOLUTION

COLOR STANDARD CARD

AOAC Official Method 999.08
An enzyme immunoassay for detection of motile and non-motile Salmonella in foods, ingredients, and environmental samples

For visual or instrumental result interpretation
**Trypticase (tryptic) broth (TSB)**

Suspend 20 g of dehydrated buffered peptone water in 1 L of deionized water. Mix thoroughly. Dispense in 225 mL aliquots for food samples. Autoclave at 121°C for 15 min.

**Pre-Enrichment:**

Universal preenrichment broth (UP)

Dissolve 38 g of dehydrated universal preenrichment broth in 1 L of deionized water. Mix thoroughly. Heat gently to dissolve completely. Dispense in 225 mL aliquots. Autoclave at 121°C for 15 min.

**Selective Enrichment:**

5X Tetrathionate broth (5XTT)

Suspend 330 g of dehydrated tetrathionate broth base in 1 L of deionized water. Mix thoroughly. Heat with agitation and boil for 1 min. DO NOT AUTOCLAVE. Cool to below 45°C and add 5 mL 1% brilliant green dye solution (see Appendix II - Pre-Enrichment). Mix thoroughly.

Buffered peptone water (BPW)

Dissolve 20 g of dehydrated buffered peptone water in 1 L of deionized water. Mix thoroughly. Dispense in 225 mL aliquots for food samples or 10 mL aliquots for environmental swabs. Autoclave at 121°C for 15 min.

**APPENDIX II - Enrichment Recipes For Processed Foods**

**Pre-Enrichment:**

Trypticase (tryptic) soy broth (TPS)

Dissolve 30 g of dehydrated Trypticase soy broth in 1 L of deionized water. Mix thoroughly. Dispense in 225 mL aliquots. Autoclave at 121°C for 15 min.

**Selective Enrichment:**

5X Rappaport-Vassiliadis R10 broth (5XRV)

Suspend 130 g of dehydrated Rappaport-Vassiliadis (RV) broth in 1 L of deionized water. Mix thoroughly. Dispense in 5 mL aliquots into large test tubes (25 X 150 mm). Autoclave at 116°C for 15 min.

5X Tetraionate broth (5XTT)

Suspend 330 g of dehydrated tetrionate broth base in 1 L of deionized water. Mix thoroughly. Heat with agitation and boil for 1 min. DO NOT AUTOCLAVE. Cool to below 45°C and add 5 mL 1% brilliant green dye solution (see Appendix II - Pre-Enrichment). Mix thoroughly. Dispense in 5 mL aliquots into sterile test tubes. Store in the refrigerator if the media will not be used within 12 h. On day of use, add 0.5 mL of iodine-iodide solution (see below) to each 5 mL test tube.

**APPENDIX III - Enrichment Recipes For Dried Powder Processed Foods**

**Pre-Enrichment:**

Brain heart infusion broth + enrichment supplement containing Oxyrase® (BHI+O)

Suspend 37 g of dehydrated brain heart infusion broth in 1 L of deionized water. Mix thoroughly. Dispense in 10 mL aliquots. Autoclave at 121°C for 15 min. On day of use, add 0.1 mL 0.1% novobiocin solution (see below) per 10 mL tube. Light sensitive - store appropriately.

**Selective Enrichment:**

5X Tetrionate broth (5XTT)

See Appendix II - Selective Enrichment

**APPENDIX IV - Enrichment Recipes For Raw Foods**

**Pre-Enrichment:**

Buffered peptone water (BPW) + novobiocin

Suspend 20 g of dehydrated buffered peptone water in 1 L of deionized water. Mix thoroughly. Dispense in 225 mL aliquots for food samples. Autoclave at 121°C for 15 min. On day of use, add 4 mL 0.1% novobiocin solution (see Appendix II - Post-Enrichment) to 225 mL BPW.

**Selective Enrichment:**

Tetrionate broth (TT)

Suspend 46 g of dehydrated tetrionate broth base in 1 L of deionized water. Mix thoroughly. Heat with agitation and boil for 1 min. DO NOT AUTOCLAVE. Cool to below 45°C and add 1 mL 1% brilliant green dye solution (see Appendix II - Pre-Enrichment). Store in the refrigerator if the media will not be used within 12 h. On day of use, add 20 mL of iodine-iodide solution (see Appendix II - Selective Enrichment). Mix thoroughly. Dispense in 10 mL aliquots into sterile test tubes.

Rappaport-Vassiliadis R10 broth (RV)

Suspend 26.6 g of dehydrated Rappaport-Vassiliadis R10 broth in 1 L of deionized water. Mix thoroughly. Dispense in 10 mL aliquots. Autoclave at 116°C for 15 min.

**Post-Enrichment:**

Trypticase (tryptic) soy broth + novobiocin (TSB+n)

See Appendix II - Post-Enrichment

**PRODUCT INFORMATION**

This product is not intended for human or veterinary use. Assurance Gold Salmonella EIA must be used as described herein. Contents of the test may be harmful if swallowed or taken internally. Do not use Assurance Gold Salmonella EIA reagents that have expired. Do not mix reagents from different Assurance kit lots.

**PRODUCT WARRANTY**

BioControl Systems, Inc. (BCS) warrants this product to be free from defects in materials and workmanship, when stored under labeled conditions and used as intended until the expiration date stated on the package. BCS agrees during the applicable warranty period to replace all defective products after return to BCS. BCS shall not have obligation under this Limited Warranty to make replacements which result, in whole or in part, from negligence of the Buyer or from improper use of the products, or use of the product in a manner for which it was not indicated. Buyer shall notify BCS of any products which it believes to be defective, at no charge. Should BCS examination not disclose any defect covered by the foregoing warranty, BCS shall so advise Buyers and dispose of the product in accordance with Buyer’s instructions.

**IF YOU NEED MORE INFORMATION ABOUT ASSURANCE GOLD SALMONELLA EIA, ITS USE OR OTHER BIOCONTROL PRODUCTS, PLEASE CONTACT:**

**Results. Right Now.**

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