TRANSIA® AG *Salmonella*

AOAC Official Method 999.08

An enzyme immunoassay for the detection of motile and non-motile *Salmonella* in foods, ingredients and environmental samples.

Intended Use

TRANSIA® AG for *Salmonella* 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 TRANSIA® AG are designed to be read either visually, using the color standard provided, or instrumentally, using a microplate reader.

Directions for use

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 Appendices 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 Oxyrase™ (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 - 26 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 Tetrathionate (5XTT) broth. Incubate 16 - 24 h in a 42°C water bath.

Dried Powder Processed Foods

Add 25 mL BHI + O to 5 mL 5X TT. Incubate 16 - 24 h in 42°C water bath.
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 TT broth. Incubate 5 - 8 h in 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 Trypticase Soy Broth+ novobiocin (TSB+n). 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+n. For dried egg products, transfer 0.2 mL of TT broth into a single tube of 10 mL prewarmed TSB+n. 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+n. Label tubes. Incubate 16 - 20 h in a 42°C water bath.

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

NOTE: *Retain original TSB+n broth under refrigeration (2–8 °C). Use for confirmation of presumptive positive results (see section E).*

Read Section **B** before proceeding. Add 0.1 mL **Reagent 1 - Extraction Reagent** to 1.0 mL TSB+n aliquot tube and vortex. Inactivate microorganisms at 100°C for 10 min. Cool tubes to 25–37°C before testing. Tubes that have been inactivated can be refrigerated at 2–8°C up to (4) days prior to testing.

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 pouch and all unused reagents at 2 - 8°C.

Wash Solution Preparation

Add 5.0 mL of **Reagent 2 - Wash Solution Concentrate** to 100 mL of deionized water. Label container. This volume is sufficient to wash (40) wells. Wash solution is stable for (30) days at room temperature. If crystals are present in wash solution concentrate, dissolve by immersing bottle in warm water for 15 min.

C. Test Procedure

a. Fit required number of microwells into holder. Reseal unused microwells in foil pouch. In addition to samples, allow (3) 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 well. Also pipette 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.

c. 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

It is acceptable to:

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-reactive wells. Avoid under filling 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)** 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 wells with the 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 Cutoff) 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 - Stop 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.*

Standardize reader by reading the BLANK well and adjusting optical density (O.D.) to zero. (2) Read sample absorbance of each well, starting with the (2) 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 manufacture's 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:

$$\frac{PC1 + PC2}{2} \times 0.25 = \text{Cutoff Value}$$

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 TRANSIA® AG *Salmonella* kit contains the following:

Test Wells
Reagent 1 - Extraction Reagent
Reagent 2 - Wash Solution Concentrate
Reagent 3 - Positive Control
Reagent 4 - Conjugate Reagent
Reagent 5 - Substrate Reagent
Stop Solution Color Standard Card

ENRICHMENT MEDIA

APPENDIX I - Recommended Pre-enrichment Media

Food Type	Enrichment Broth
Nonfat dry milk	Brilliant green dye water
Liquid egg products	Trypticase (tryptic) soy broth
Chocolate based products	Reconstituted nonfat dry milk + brilliant green dye
Orange juice	Universal Pre-enrichment broth
Dried powder processed products	Brain heart infusion broth + enrichment supplement containing Oxyrase®
Raw foods	Buffered peptone water + novobiocin
All other foods	Buffered peptone water

APPENDIX II - Enrichment Recipes for Processed Foods

Pre-Enrichment:

Brilliant green dye water

Add 2 mL 1% brilliant green dye solution (see below) per 1 L sterile water.

1% Brilliant green dye solution

Dissolve 1 g brilliant green dye in 100 mL sterile water. **DO NOT AUTOCLAVE.**

Reconstituted nonfat dry milk + brilliant green dye

Dissolve 100 g nonfat dry milk in 1 L deionized water. Mix thoroughly. Autoclave at 121°C for 15 min. Cool and add 2.0 mL 1% brilliant green dye solution (see above). Mix thoroughly.

Buffered peptone water (BPW)

Suspend 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.

Universal pre-enrichment 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.

Trypticase (tryptic) soy broth (TSB)

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 133 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 Tetrathionate broth (5XTT)

Suspend 230 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. 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.

Iodine-iodide solution

Dissolve 6 g iodine and 5 g potassium iodide in 20 mL sterile water. **DO NOT AUTOCLAVE.**

Post-Enrichment:

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

Dissolve 30 g of dehydrated trypticase (tryptic) soy 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.

0.1% Novobiocin solution

Dissolve 0.1 g novobiocin (sodium salt) in 100 mL deionized water. **DO NOT AUTOCLAVE.** Filter through 0.2 nm filter. Store at 4°C· Light sensitive - store appropriately.

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 brain heart infusion broth in 1 L of deionized water. Mix thoroughly. Autoclave at 121°C for 15 min. On day of use, after sample addition and blending, add 1 mL of enrichment supplement containing Oxyrase® to each 225 mL aliquot. Mix gently.

Selective Enrichment:

5X Tetrathionate broth (5XTT)

See Appendix II - Selective Enrichment

Post-Enrichment:

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

See Appendix II - Post-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:

Tetrathionate broth (TT)

Suspend 46 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 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. TRANSIA® AG *Salmonella* must be used as described herein. Contents of the test may be harmful if swallowed or taken internally. **Do not use TRANSIA® AG *Salmonella* reagents that have expired. Do not mix reagents from different TRANSIA® AG kit lots.**

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.

BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

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 during the warranty period. At BCS option, such products shall be returned to BCS, transportation and insurance prepaid. BCS shall replace any such product found 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.

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