INTENDED USE

TRANSIA® ADDITIVE Salmonella Gold 24h (TAG 24) is intended for use in combination with TRANSIA PLATE Salmonella Gold (SA0180 or SA0190) for the detection of Salmonella genus using a specific enrichment procedure within 24 hours. The assay reliably recovers and detects low levels of Salmonella in a variety of foods and feeds.

The TAG 24 Salmonella method has been certified by NF Validation for all foods and feeds.

ASSAY PRINCIPLE

The rapid enrichment (22-25 h) is based on a proprietary enrichment protocol using buffered peptone water (BPW) and brain heart infusion (BHI) as enrichment media, both supplemented by the TAG 24.

MATERIALS

**Kit components**
- TAG 24 – 4 x 30 mL

**Equipment required but not provided**

For sample and reagent preparation:
- Analytical balance and weighing vessels
- Homogeniser or mixer (stomacher)
- Stomacher bags, preferably with a filter (400 mL)
- Tubes (20 mL) for the subcultures
- Magnetic stirrer
- Air incubator at 37°C ± 1°C
- Air incubator at 41.5°C ± 1°C or water bath with circulating water at 41.5°C ± 0.5°C
- Bath with boiling water (95 - 100°C)
- Vortex
- Tubes 1L, beaker 1L
- Distilled water
- Buffered peptone water
- Brain heart infusion

For the immunoenzymatic test:
- TRANSIA PLATE Salmonella Gold (please refer to package inserts of the kits)

For confirmation of samples:
- Selective agar plates such as xylose lysine deoxy- cholate agar (XLD), brilliant green agar (BGA), or a Salmonella chromogenic agar.
- Biochemical identification gallery.

STORAGE CONDITIONS

The kit components should be stored at 2 – 8 °C.

SAFETY

Good laboratory practice (refer to EN ISO 7218) should be employed when using this method. Safety clothing should be worn and skin contact with reagents avoided. Do not ingest. Safety data sheets are available on request.

TEST PROCEDURE

**Preparation of reagents**

TAG 24 is provided ready-to-use.

Shake each vial manually or with a vortex before use.

Do not interchange reagents between kits with different batch numbers.

**Preparation of the buffered peptone water, brain heart infusion enrichment broth and plating media:**

Follow the manufacturer’s instructions.

Prepare the enrichment broths by adding TAG 24 supplement the BPW and the BHI. (for the actual concentrations refer to Preparation of samples below). Shake the enrichment broth bottle after addition of the TAG 24.

Supplemented broths can be stored up to 5 days at 3 °C ± 2 °C before use.

**Preparation of samples**

1. Homogenize in a stomacher bag X grams or X mL of the sample with 9X mL of buffered peptone water supplemented with TAG 24, at a concentration of 5 mL/L (meaning 1.125 mL for 225 mL).

**Note:** Samples exceeding 25 g have not been included in the NF Validation.

2. Incubate for 18-20 hours at:
   - 41.5°C ± 1°C (meat and meat-based products)
   - 37°C ± 1°C (all other products).
3. Homogenize and dispense 1 mL of the pre-culture broth in 10 mL of BHI supplemented with 50 μL of TAG 24.

4. Incubate for 4-5 hours at 41.5°C ± 1°C in an air incubator, or preferably at 41.5°C ± 0.5°C in a water bath with circulating water.

5. In a tube, heat 1 to 2 mL of the enrichment broth in boiling water for 20 minutes, then cool to room temperature.

Keep the rest of the tube with the non-heated enrichment broth available if confirmation of a positive or doubtful ELISA result is necessary. Keep this non-heated broth at 41.5 ± 1 °C during the ELISA test.

6. Perform the immunoenzymatic test using the TRANSIA PLATE Salmonella Gold procedure (refer to TRANSIA PLATE Salmonella Gold package insert).

**Storage of samples**

If the test cannot be performed immediately after the BHI + TAG 24 incubation time (4 to 5 hours), or before performing the confirmation of positive or doubtful samples, incubated BHI + TAG 24 (before heat inactivation) can be stored up-to 48 hours at 3°C ± 2 °C. The non-deleterious effect of this additional storage has been validated during the AFNOR validation study.

**Sample preparation flow chart (example)**

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X g or X mL of sample to
9X mL BPW + TAG 24 (5 L/L
41.5 ±1°C (meat) or 37±1°C(other matrices) for 18 – 20 h

1 mL

10 mL BHI + 50µL TAG 24 at 41.5 ±1°C for 4 – 5 h

1.2 mL

Heat inactivation, 95-100 °C; 20 min

TRANSIA® PLATE Salmonella Gold
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**CONFIRMATION OF POSITIVE RESULTS**

A positive result shall be confirmed by streaking of the enrichment broth (BHI + TAG 24) onto selective media plates followed by a biochemical identification (see EN ISO 6579:2002)

If the positive TRANSIA PLATE Salmonella Gold result is not confirmed, those working according to the protocol approved by NF Validation are requested to guarantee that all possible actions have been taken in order to validate the final result.

We recommend using additional media plates utilizing other biochemical features of the Salmonella genus. In the case of meat or meat-based products analysis, the confirmation of positive results can sometimes be difficult because of the background flora. During the NF validation study, it was demonstrated that doing a subculture (0.1 mL) of the BHI + TAG 24 broth in a Rappaport Vassiliadis Soya broth (RVS) could be an efficient solution.

**Detection limit**

The NF validation study showed that the detection limit of the method was between 0.2 and 1.5 cells/25 grams of sample.

**Specificity**

During the NF validation study inclusivity was evaluated with 50 Salmonella strains, all detected with the TAG 24 method. 30 non-Salmonella strains tested in the exclusivity study did not show any cross reaction.

**Note:** The enhanced enrichment may damage bacterial cells in pure culture if sample matrix is not present. It is recommended to evaluate the inclusivity of the method by cultivating Salmonella strains in a food matrix (i.e. using 25 mL UHT milk + 225 mL BPW + TAG 24).
AFAQ AFNOR Certification has granted NF Validation Mark to TAG 24 Salmonella method, which combines use of TRANSIA ADDITIVE Salmonella Gold 24h supplement and TRANSIA PLATE Salmonella Gold ELISA test. Validation scope: All human and animal food products. Reference method EN ISO 6579:2002. Validation study performed according to the EN ISO 16140 standard.

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 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.