**AGROINDUSTRIAL STRENGTHENING OF QUINOA PROJECT THROUGH THE SCALING OF PROTOTYPES IN RELEVANT ENVIRONMENTS FOR THE INDUSTRY IN THE DEPARTMENT OF CAUCA**

**DETERMINATION OF SALMONELLA**

**Guide Code: 001**

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1. **AIM**

Describe methodology used to determine and quantify the presence of *Salmonella spp* based on NTC 4574

1. **BASIS**

*Salmonella spp* is a Gram-negative bacterium, which forms typical colonies on a selective medium. (NTC 4574, 2007) It can be found in foods such as chicken, beef, pork, eggs, fruits, vegetables, and even processed foods.

It is the etiological agent of Salmonellosis, one of the foodborne diseases (ETA) with important repercussions on public health.(Castañeda-Salazar et al., 2017)

1. **MATERIALS**

|  |  |
| --- | --- |
| **MATERIAL** | **AMOUNT** |
| Petri boxes | 3 |
| Spatula | 1 |
| Glass clock | 3 |
| Blue tips 1 mL | 1 |
| erlemeyer | 3 |
| glass tubes | According to number of replicates |

Note: Wash, dry and sterilize materials.

1. **REAGENTS**

|  |  |
| --- | --- |
| **selective media** | **Quantity** |
| Buffered peptone water | According to supplier specifications |
| [Rappaport Vassiliadis Broth (RVA)](https://legacy.bd.com/europe/regulatory/assets/ifu/hb/ce/ba/es-ba-257257.pdf) | According to supplier specifications |
| Muller Kauffmann Tetrathionate- | According to supplier specifications |
| XLD (Xylose, Lysine, Deoxycholate) agar | According to supplier specifications |
| Salmonella Shigella (SS) Agar | According to supplier specifications |

1. **TEAMS**

Clearly and descriptively detail the equipment and quantity for the development of the method or determination.

|  |  |
| --- | --- |
| **Team** | **Quantity** |
| Autoclave | one |
| Incubator | one |
| Kiln | one |
| micropipette 1000μL | one |
| micropipette 100μL | one |
| biosafety cabinet | one |

1. **PROCESS**
   1. **Pre-enrichment in non-selective liquid medium**

A buffered solution is inoculated with peptone water or lactose broth with 25 g of sample, incubated at 37°C±1 °C for 18h + 2h

* 1. **Enrichment in the selective medium**

0.1 mL of the culture medium obtained in numeral 6.1 is transferred to a glass tube containing 10 mL of the RSV medium.

In another tube, 1 mL of the culture medium obtained in numeral 6.1 containing 10 mL of the MKTTn medium is transferred.

RSV broth is incubated at 41.5 for 24 h.± 3 h and the MKTTn broth is incubated at 37 °C for 24 h ± 3 h.

* 1. **Sowing in selective medium**

After incubating 24 h± 3h, using the culture obtained in the RVS broth. The surface of the Petri dish containing the first selective plating medium (XLD agar) is seeded using a loop. The same medium obtained in the RVS broth is also seeded on salmonella shigella (SS) agar so as to obtain isolated colonies.

The same is done with the other selective medium MKTTn.

The inoculated dishes are inverted, and placed to incubate at 37°C.± 1°C for the first and second half. It is examined after 24 ± 3 hours or according to the culture medium manufacturer's instructions, if necessary, after 48 hours to verify the presence of colonies which, based on their characteristics, are considered presumptive to be Salmonella spp.

* 1. **confirmation test**

Colonies of Salmonella spp. Isolated presumptive as described in numeral 6.3. and confirmed by appropriate biochemical and serological assays

1. **DISPOSAL OF CHEMICAL AND/OR BIOLOGICAL WASTE.**

After the presence or absence of Salmonella spp, the boxes are deactivated in an autoclave. The medium is then thrown into a bag indicating biohazard.

**BIBLIOGRAPHY**

Colombian technical standard (2018) Microbiology of food and animal feed. Horizontal method for the detection of Salmonella spp. (NTC 4574)

Castañeda-Salazar, R., Del Pilar Pulido-Villamarín, A., Mendoza-Gómez, MF, Carrascal-Camacho, AK, & Sandoval-Rojas, KL (2017). Salmonella spp. Isolation and identification in eggs for human consumption from different urban areas in Bogotá, Colombia, 2015. Infectio, 21(3), 154–159. https://doi.org/10.22354/in.v21i3.672