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

**Identification of total coliforms NTC 4458**

**Guide Code: 001**

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1. **AIM**

Determine and quantify the presence of coliforms and Escherichia coli against a chromogenic medium

1. **BASIS**

Coliforms: group of bacteria that have certain characteristics in common and relevant importance as indicators of contamination of water and food. They are commonly found in plants, animals, including humans. The group of coliforms is made up of the following genera: Escherichia spp, Klebsiella spp, Enterobacter spp and Citrobacter spp, among others.

Principle of the method: when the enzymatic technique is used, Escherichia coli is defined as any bacterium that gives a positive response to the coliform group and also possesses the enzyme B-Glucuronidase, which has the property of opening the fluorogenic substrate, resulting in the fluorogen release.

A fluogenic substrate such as 4-methyl-umbelliferyl-β-D-glucuronide (MUG)It is used to detect the enzyme B-glucuronidase, produced by E coli.

The culture medium used is based on the defined substrate technology, which uses nutrients - indicators that produce color, fluorescence, or both, when metabolized by coliform and E coli microorganisms; which are highlighted simultaneously.

The reagent provides two specific nutrient-indicators: ONPG (O-nitrophenyl-β-d-galactopyranoside) and MUG (4-methyl-umbelliferyl-β-d-glucuronide). During the metabolization of these nutrients by the Coliform enzyme-β-D galactosidase and-β-glucuronidase*e coli*, a yellow color (from the ONPG) and fluorescence (from the MUG) are produced. with which the presence of coliforms and E coli is confirmed. respectively.

Seedings are carried out using a selective culture medium and using a representative and specific amount of the test sample if the initial product is liquid, or a specific amount of an initial suspension in the case of other products. Other seedings are carried out under the same conditions, using tenfold dilutions of the test sample in the initial suspension.

1. **MATERIALS**

| **MATERIAL** | **AMOUNT** |
| --- | --- |
| Petri boxes | 3 |
| Spatula | one |
| Glass clock | 3 |
| Blue tips 1 mL | one |
| erlemeyer | 3 |

Note: Wash, dry and sterilize materials.

1. **REAGENTS**

| **agar** | **Quantity** |
| --- | --- |
| E. coli chromogenic. | 4.15g |
| Distilled water | 150mL |

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 |
| shaker | one |
| Colony count equipment | one |
| micropipette 1000 μL | one |
| Laminar flow cabinet | one |

1. **PROCESS**
   1. **Preparation of sample**

Mix 2 g of sample with 18 mL of distilled water in an Erlenmeyer, shake and allow to settle. Repeat procedure with the number of samples. Depending on the initial count, dilutions are prepared according to the NTC 4491-1 standard.

* 1. **agar preparation**

The agar is prepared according to the specifications on the package's technical data sheet. For 150 mL, 4.15 g of coinstant chromogenic agar are weighed, distilled water is added and later it is left to boil on a heating plate with constant stirring. Later, it is sterilized for 20 min at 259 °C and 20 psi.

* 1. **Process**
     1. **Inoculation and incubation**

In three previously sterile Petri dishes. using a micropipette1000 μL of sample of the first decimal dilution (10-1) is transferred.

The described procedure is repeated with the additional dilutions, using a micropipette for each decimal dilution.

Subsequently, between 10 mL to 15 mL of specific medium are added to each Petri dish. Carefully mix the inoculum with the medium and allow it to solidify.

The prepared dishes are inverted and incubated at 35 °C ± °C for 24 h ± 2 h.

* + 1. **colony count**

After the specified period for incubation, select Petri dishes containing no more than 1501 colonies. Count the typical and non-typical colonies in each box, using the colony counting kit.

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

After the determination of E.coli, 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 enumeration of coliforms or Escherichia coli or both. Counting technique using fluorogenic or chromogenic media (NTC 4458)

**ANNEXES**