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

**Identification of *Staphylococcus aureus* NTC 4779**

**Guide Code: 005**

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1. **AIM**

Describe the methodology used to determine the presence of Staphylococcus aureus NTC 4779

1. **BASIS**

*staphylococcus*aureus:Compared to other microbial pathogens, *S. aureus* is more dangerous due to toxin production, the ability to form colonies in a variety of environments (non-pathogenic and those of clinical importance), the ability to change metabolic rates (for example , cell wall modifications, population adaptations, cytoplasmic modulations) according to the prevailing conditions and high capacity to overcome the host defense system(Hussain Chan et al., 2021). It can survive for a long time in acidic or alkaline medium (4.5 to 8 pH), high salt concentration (up to 15%), low and high temperature (4 to 45 °C) and dry conditions.(Onyango & Alreshidi, 2018). Common foods affected by S. aureus include red meat, canned foods, poultry products, dairy products, sauces, and cream-filled bakery items; S. aureus is characterized by the production of extracellular (nuclease, lipase, coagulase, hemolysins) and enterotoxins (a group of water-soluble, heat-resistant, heterogeneous single-chain globular protein responsible for food poisoning).(Hussain Chan et al., 2021).

1. **MATERIALS**

|  |  |
| --- | --- |
| **MATERIAL** | **AMOUNT** |
| Petri boxes | 10 |
| Spatula | 1 |
| Glass clock | 3 |
| Rack (100 tips) tips 1000μL | 1 |
| Rack (100 tips) tips 100μL | 1 |
| erlemeyer | 3 |
| Glass beads | 1 |

Note: Wash, dry and sterilize materials.

1. **REAGENTS**

|  |  |
| --- | --- |
| **agar** | **Quantity** |
| Baird Parker | According to supplier specifications |
| Distilled water | - |

1. **TEAMS**

|  |  |
| --- | --- |
| **Team** | **Quantity** |
| Autoclave | 1 |
| Incubator | 1 |
| Kiln | 1 |
| shaker | 1 |
| Colony count equipment | 1 |
| micropipette 1000μL | 1 |
| micropipette 100μL | 1 |
| Laminar flow cabinet | 1 |

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

Mix 10 g of sample with 90 mL of distilled water in an Erlenmeyer, shake in a shaker.(MaxQ 4450 orbital Thermo Ficher Scientific USA) for 10 min at 150 rpmand let 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**

Baird Parker Agar is prepared according to the specifications on the container's data sheet. Weigh quantity (g) of Baird Parker agar, then add to volume of distilled water;then it is left to boil on a heating plate with constant stirring. Later, it is sterilized in an autoclave for 20 min at 259 °C and 20 psi. Once sterile, it is served in previously sterile boxes and is allowed to solidify, in a contamination-free environment.

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

Using a micropipette, transfer1000 μL of sample from the first decimal dilution (10-1) to the next (10-2); the described procedure is repeated with the additional dilutions, using a different tip for each decimal dilution.

pour agarBaird Parkerin Petri dishes so that it gels, once the gelation is completed, an aliquot of 100 μL of the dilutions is taken and deposited in the agreed Petri dishes for repetitions, with the help of glass beads shake vigorously in the Petri dish, repeat the process with each of the repetitions.

The solidified Petri dishes are inverted and packed in vinyl and incubated at 35 °C and 24 h. Monitor during the incubation of the microorganism.

* + 1. **colony count**

After the specified period for incubation, select the Petri dishes containing colonies ≤ 300 colonies and count them in the colony counting equipment, using dim light, taking care to mark the counted colonies, to avoid confusion.

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

Finished determination of*staphylococcus*aureus, the boxes must be deactivated in an autoclave. The medium is then thrown into a bag indicating biohazard.

**BIBLIOGRAPHY**

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Onyango, LA, & Alreshidi, MM (2018). Adaptive Metabolism in Staphylococci: Survival and Persistence in Environmental and Clinical Settings. Journal of Pathogens, 2018(Cm), 1–11. https://doi.org/10.1155/2018/1092632