9.DETECTION OF *SALMONELLA*

SCOPE:

To detect *Salmonella* in Coconut milk and Desiccated Coconut products.

PRINCIPLE:

Salmonella are facultative anaerobic,Gram-negative, non-spore-forming, rod shaped and predominantly motile enterobacteria. Measured aliquots of samples are preenriched in non-selective enrichment medium(BPW) and incubated at 37 ± 1ºC for 16 h to 20 h. After incubation measured aliquots are inoculated into selective enrichment medium (RV medium & SC medium) and incubated RV medium at 42 ± 1ºC for 24 h and SC broth at 37 ± 1ºC for 24 h. After incubation sub cultures are made on BGA and XLD and incubated at 37 ± 1ºC for 24 h to 48 h. The presence of *Salmonella in the* sample is determined by observing the characteristic colonies developed on the plates.

DILUENT, CULTURE MEDIA AND REAGENTS :

- Buffered peptone water (BPW)
- Rappaport-Vassiliadis medium (RV)
- Selenite Cystine medium (SC)
- Brilliant green agar (BGA)
- Xylose lysine deoxycholate agar (XLD)

APPARATUS, INSTRUMENTS AND GLASSWARES:

- Laminar airflow chamber
- Autoclave
- Hot air oven
- Sterile petri plates
- Weighing balance
- Dilution bottles
- Water bath
- Screw cap tubes
- Sterile spatula
- Micropipette
- Sterile tips

PREPARATION OF TEST SAMPLE:

1.Desiccated coconut

All the samples are aseptically mixed in order to make the sample uniform.

2. Coconut milk

All the samples are aseptically mixed in order to make the sample uniform

PROCEDURE:

- Aseptically transfer 25g of sample into 225 ml of sterile BPW in screw cap bottles (For specific customer requirements increase the sample quantity with BPW in the ratio of

  1: 9 e.g. 375g sample into 3375ml sterile BPW). Mix the sample by shaking 25 times in 7 seconds with a 1 foot(30cm) movement. For swab samples add 10 ml swab samples to 90 ml of sterile BPW and mix well.
- Incubate the bottles at 37 ± 1ºC for 16 to 20 h.
- After incubation transfer 10ml sample to 100ml SC medium and 0.1ml sample to 10ml RV medium.
- Incubate SC medium at 37 ± 1ºC for 24 h and RV medium at 42 ± 1ºC for 24 h.
- After incubation streak out a loopful sample from SC medium and RV medium on to BGA and XLD agar.
- Incubate the plates at 37 ± 1ºC for 24 to 48 h.
- After incubation observe the plates for the presence of *Salmonella* colonies (Pinkish white colonies on BGA and pink colonies with black center on XLD).
- If there is any typical colonies, sub culture on Nutrient agar plates and do biochemical confirmation using HI Salmonella identification kit.

SEROLOGICAL CONFIRMATION:

- Place three separate loopfuls of normal saline (0.85% sodium chloride) on a clean glass slide.
- Take a small part of a suspect *Salmonella* colony from an overnight nutrient agar plate

  and mix thoroughly with both drops of normal saline on the slide to obtain a smooth suspension.
- Add one drop of Salmonella Poly O & Vi antisera to one bacterial suspension

and

Salmonella Poly H antisera to second bacterial suspension drops on the slide, to the other (control) add one loopful of normal saline.

- Mix the antiserum with the bacterial suspension using a sterile loop.
- Gently tilt the slide back and forth for one minute and observe for agglutination under normal lighting conditions.

EXPRESSION OF RESULT:

Report result as Present/Absent of *Salmonella*/tested volume of the sample.

REFERENCE:

IS 5887 (Part 3) : 1999 Reaffirmed 2009 / ISO 6579 : 1993