

	<b>HOLISTA TRANZWORLD PRIVATE LIMITED</b> 2/91, MARAVANMADAM, ANTONIYARPURAM, TUTICORIN -628 101	Doc No : HTPL-SOP22
	<b>Food Safety and Quality Management System</b>	Issue/Rev : 1.0
	<b>Lab Test Procedure SOP</b>	Date : 19.09.2022

## 8.DETECTION OF *ESCHERICHIA COLI*

### SCOPE:

To detect *Escherichia coli* in Coconut milk and Desiccated Coconut products.

### PRINCIPLE:

The typical *E.coli* is aerobic, Gram-negative rod, motile, fermenting lactose with the production of gas. Measured aliquots of sample or sample dilutions are inoculated into MacConkey broth medium and incubated at  $37 \pm 1^\circ\text{C}$  for 18 h to 24 h. After incubation sub cultures are made on Eosin methylene blue agar and incubated at  $37 \pm 1^\circ\text{C}$  for 18 h to 24 h. The presence of *E.coli* in the sample is determined by observing the characteristic colonies developed on the plates.

### DILUENT, CULTURE MEDIA AND REAGENTS :

- Peptone salt water
- Macconkey broth
- Eosin methylene blue agar (EMB agar)
- Tergitol-7 agar
- Macconkey agar

### 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.

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## 2. Coconut milk

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

### PROCEDURE:

- Aseptically transfer 10 gm of sample into 90 ml of diluent in dilution bottles. Mix the sample by shaking 25 times in 7 seconds with a 1 foot(30cm) movement. This will give  $10^{-1}$  dilution.
- Using sterile pipette transfer 10ml sample from  $10^{-1}$  dilution into 10ml of double strength MacConkey broth medium.
- Incubate the tubes at  $37 \pm 1^{\circ}\text{C}$  for 18 to 24 h.
- After incubation observe for growth with fermentation of lactose (yellow colour and gas production) in the MacConkey broth medium and streak out a loopful on to EMB agar, Tergitol-7agar, Macconkey agar.
- Incubate the plates at  $37 \pm 1^{\circ}\text{C}$  for 18 to 24 h.
- After incubation observe the plates for the presence of typical *E.coli* colonies (dark violet colonies with black center and with green metallic sheen).
- Incubate Tergitol -7 agar plate at  $37^{\circ}\text{C}$  for 24 hours yellow colonies with deep yellow halo colonies will appear.
- Incubate Macconkey agar plate at  $37^{\circ}\text{C}$  for 24 hours Lactose fermenting colonies with pink in colour.

### EXPRESSION OF RESULT:

Report result as Present/Absent of *E.coli*/gm of the sample.

### REFERENCE:

IS 5887 (Part 1) : 1976 Reaffirmed 2009, ISO 9308:1:2014