

	<b>HOLISTA TRANZWORLD PRIVATE LIMITED</b> 2/91, MARAVANMADAM, ANTONIYARPURAM, TUTICORIN -628 101	<b>Doc No : HTPL-SOP17</b>
	<b>Food Safety and Quality Management System</b>	<b>Issue/Rev : 1.0</b>
	<b>Lab Test Procedure SOP</b>	<b>Date : 19.09.2022</b>

## 2. ENUMERATION OF COLIFORMS

### SCOPE:

To enumerate coliforms in raw materials, Coconut milk and Desiccated Coconut products by counting the colonies growing in a solid medium after incubation at  $37 \pm 1^\circ\text{C}$ .

### PRINCIPLE:

Coliforms are indicator organisms associated with poor hygienic processing conditions. The coliform group are aerobic and facultative anaerobic gram negative, non-sporeforming rod shaped bacteria which ferments lactose with gas formation. Measured aliquots of sample or sample dilutions are mixed with violet red bile agar (VRBA). Plates are incubated at  $37 \pm 1^\circ\text{C}$  for 24 hrs. The number of coliforms per ml or gm of a sample is determined by counting the characteristic colonies developed in the plates.

### DILUENT, CULTURE MEDIA AND REAGENTS:

- Peptone salt water
- Violet red bile agar (VRBA)

### APPARATUS, INSTRUMENTS AND GLASSWARES:

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

### PREPARATION OF TEST SAMPLE:

#### 1. Desiccated coconut

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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 10 ml/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.
- Aseptically transfer 1 ml sample from  $10^{-1}$  dilution into a tube containing 9 ml dilution blank and mix well. This result in  $10^{-2}$  dilution. Accordingly make serial dilutions depending on the expected count of the product tested.
- Pipette 1ml of each dilution into a separate, duplicate and appropriately marked petri plates. (For liquid products (eg. Milk) direct sample can also be plated). (For Butter transfer 10 ml sample from  $10^{-1}$  dilution into two plates (5ml+5ml).
- Use a new sterile pipette for each decimal dilution.
- Pour about 15 ml of violet red bile agar at  $44^{\circ}\text{C}$  to  $47^{\circ}\text{C}$  into each plate within 15 minutes of initial dilution preparation.
- Carefully mix the inoculum with the medium by rotating the petri plates and allow the mixture to solidify on a cool horizontal place.
- Prepare a control plate with medium for checking the sterility.
- After solidification, pour about 4 ml of VRBA at  $44^{\circ}\text{C}$  to  $47^{\circ}\text{C}$  on to the surface of the inoculated medium to prevent the spreading colonies and allow to solidify.
- After solidification invert the plates and incubate at  $37 \pm 1^{\circ}\text{C}$  for  $24 \pm 2$  h

### COUNTING OF COLONIES:

After incubation count the characteristics colonies (purplish red due to lactose fermentation having a diameter of 0.5mm or greater and sometimes surrounded by a reddish zone of precipitated bile) on each plate containing not more than 150 colonies.

### EXPRESSION OF RESULT:

The number of coliforms per gram or per milliliter of sample is equal to:

$$\frac{\sum c}{\text{-----}}$$

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$$(n_1 + 0.1n_2) d$$

Where

$\Sigma c$  = is the sum of the colonies counted on all the plates;  
 $n_1$  = is the number of plates retained in the first dilution;  
 $n_2$  = is the number of plates retained in the Second dilution;  
 $d$  = is the dilution factor corresponding to the first dilution.

Round the result calculated to two significant figures.

Report result as number of coliforms CFU /gm or ml of the sample.

If there were no colonies on plates from the initial suspension, if the initial product was solid, the number of coliforms per gram of product should be reported as fewer than 10 (<10) cfu/gm.

If there were no colonies on plates from the initial suspension, if the initial product was liquid, the number of coliforms per milliliter of product should be reported as less than 1(<1) cfu/ml.

For butter count all the characteristic colonies on two plates and report as number of coliforms CFU /gm

#### REFERENCE:

IS 5401(Part 1):2012/ ISO 4832: 2006

IS 3507: 1966