1. STANDARD PLATE COUNT

SCOPE:

To enumerate the microorganisms in raw materials, Coconut milk and Desiccated Coconut products by counting the colonies growing in a solid medium after aerobic incubation at 37 ± 1ºC.

PRINCIPLE:

Measured aliquots of sample or sample dilutions are mixed with standard plate count agar. Plates are incubated at 37 ± 1ºC for 48 ± 3 hrs. The number of microorganisms per ml or gm of a sample is determined by counting the colonies developed on the plates.

DILUENT, CULTURE MEDIA AND REAGENTS:

- Peptone salt water
- Plate count agar

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**

   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).
- Use a new sterile pipette for each decimal dilution.
- Pour about 15 ml of plate count agar at 44ºC to 47ºC into each plate. The time elapsing between the end of the preparation of the initial suspension (10^{-1}) and the moment when the medium is poured into the plates shall not exceed 45 min.
- Carefully mix the inoculum with the medium by rotating the Petri plates and allow the mixture to solidify on a cool horizontal surface.
- After solidification invert the plates and incubate at 37 ± 1ºC for 48 ± 3 hrs. Do not stack the plates more than six high. Stacks of plates should be separated from one another and from the walls and top of the incubator.
- After incubation count the colonies in each plate using colony counter or under subdued light. It is important that pinpoint colonies should be included in the count, avoid mistaking particles of undissolved or precipitated matter in plates for pinpoint colonies. Spreading colonies shall be considered as single colonies. If less than one-quarter of the dish is overgrown by spreading, count the colonies on the unaffected part of the plate and calculate the corresponding number of the entire plate. If more than one-quarter is overgrown by spreading colonies, discard the plates.

EXPRESSION OF RESULT:

1. General case - Plates containing between 15 and 300 colonies.

Retain plates containing not more than 300 colonies at two consecutive dilutions .It is necessary that one of these plates contains at least 15 colonies.

Calculate the number ***N*** of microorganisms per milliliter or per gram of sample, depending on the case, using the following equation.

$$N = \frac{\sum c}{(n_1 + 0.1 n_2)\, d}$$

Where

Σc = is the sum of the colonies counted on all the plates retained;

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 CFU /gm or ml of the sample.

2. Estimation of small numbers

If the two plates corresponding to the test sample (liquid products) or the initial suspension (other products), contain less than 15 colonies, calculate arithmetic mean ***m*** of the colonies counted on both plates.

Report the result as follows,

Estimated number of microorganisms per milliliter

$N_E = m$ CFU/ml (Liquid products)

Estimated number of microorganisms per gram

$N_E = m \times d^{-1}$ CFU/gm (other products), where ***d*** is the dilution factor of the initial suspension.

3. No colonies

If the two plates corresponding to the test sample (liquid products) or the initial suspension (other products) contain no colonies, report the result as follows,

Less than 1 CFU/ml (liquid product)

\ Less than 1 x d $^{-1}$ **CFU**/gm (other products), where ***d*** is the dilution factor of initial suspension.

REFERENCE:

IS 5402: 2012 / ISO 4833 : 2003.