

Dairy Science & Technology

(Animal Husbandry and Dairying)

E –Content

Microbiological Test (S.P.C)

by

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E –Content(Pre Ph D Course work)

(Dairy Science &Technology (Milk and Milk product Technology))

Microbiological Test -Standard Plate Count(S.P.C)

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1. Introduction-The Standard Plate Count (SPC), also known as the **Aerobic Plate Count (APC)** or **Total Viable Count (TVC)**, is a widely used microbiological method for determining the total number of **viable aerobic microorganisms** present in milk and dairy products. It provides an estimate of the microbial load and serves as an important indicator of the hygienic quality of food products. A high SPC value generally indicates poor sanitation, contamination during handling, improper processing, or inadequate storage conditions.

2. Principle-The principle of SPC is based on the ability of viable microorganisms to grow and multiply on a suitable nutrient medium under favorable conditions of temperature and time. Each viable bacterial cell or a group of cells develops into a visible colony. After incubation, the colonies are counted and the results are expressed as **Colony Forming Units per milliliter (CFU/mL)** for liquid samples or **CFU per gram (CFU/g)** for solid samples.

3. Objectives -To estimate the total viable bacterial population in a sample.

- To assess the hygienic quality of milk and dairy products.
- To evaluate the effectiveness of cleaning and sanitation procedures.
- To monitor the efficiency of processing and storage conditions.
- To determine the shelf-life and keeping quality of food products.

4. Materials and Equipment Required-Sterile Petri dishes

- Sterile pipettes or micropipettes
- Plate Count Agar (PCA)
- Dilution blanks (0.1% peptone water or sterile saline solution)
- Test tubes and dilution bottles
- Incubator

- Colony counter
- Water bath for melting agar
- Marker and laboratory accessories

5. Procedure/Method-A. Sample Preparation

The milk sample is thoroughly mixed to ensure uniform distribution of microorganisms before testing.

B. Serial Dilution -1. Transfer 1 mL of milk into 9 mL of sterile diluent to prepare a 10^{-1} dilution.

2 Transfer 1 mL from the 10^{-1} tube into another 9 mL of diluent to obtain a 10^{-2} dilution.

3 Continue the process to obtain further dilutions such as 10^{-3} , 10^{-4} , etc

C. Pour Plate Method-1 Transfer 1 mL of an appropriate dilution into a sterile Petri plate.

2. Add approximately 10–20 mL of melted Plate Count Agar cooled to about 45°C .

Mix gently by rotating the plate to distribute the microorganisms evenly.

4. Allow the agar to solidify.

D. Incubation

- Invert the plates and incubate them at $35 \pm 1^{\circ}\text{C}$ for 48 ± 2 hours (according to standard methods).
- During incubation, visible colonies develop from viable microorganisms.

E. Colony Counting

After incubation:

- Select plates containing 25–250 colonies.
- Count the colonies using a colony counter or manually.
- Record the number of colonies.

6. Calculation of SPC

Formula: $t\{\text{SPC (CFU/mL)}\} = \{\text{Number of colonies}\} * \{\text{dilution}\}$

Example:

Suppose a plate prepared from the **10^{-4} dilution** gives **180 colonies**.

$$\{\text{SPC}\} = 180 * 10000$$

$$\Rightarrow 1800000 \text{ CFU/mL}$$

Therefore, the microbial count in the original milk sample is:

$$\text{SPC} = 1800000/\text{ml}$$

7. Interpretation of Results

- **Low SPC:** Indicates good hygienic practices, proper processing, and fresh quality.
- **High SPC:** Indicates contamination, poor sanitation, improper handling, or unsuitable storage conditions.

8. Factors Affecting SPC in Milk

- Hygiene during milking
- Cleanliness of milking equipment and utensils
- Quality of water used for cleaning
- Health status of the animal
- Speed of cooling after milking
- Storage temperature and duration
- Handling and transportation conditions

9. Applications of SPC

- Quality control in dairy plants
- Assessment of raw milk quality
- Monitoring effectiveness of pasteurization and sanitation programs
- Evaluation of shelf life of milk and dairy products
- Microbiological examination of foods and beverages

10. Advantages

- Simple and economical method.

- Provides an estimate of viable bacterial load.
- Useful for routine quality control.
- Standardized and widely accepted test.

11. Limitation

- Counts only viable microorganisms capable of growing under the test conditions.
- Some bacteria may not grow on Plate Count Agar.
- Requires 24–48 hours for results.
- Does not identify specific microorganisms or pathogens.
- Colonies may arise from a cluster of cells rather than a single cell

12. Conclusion

The **Standard Plate Count (SPC) test** is a fundamental microbiological technique used to evaluate the microbial quality and sanitary condition of milk and dairy products. It estimates the total number of viable aerobic bacteria and is an essential tool in dairy quality assurance.