MICROBIOLOGICAL ANALYSIS
OF FOOD & FOOD PRODUCTS

By
Angshuman Saha
Assistant Director
Export Inspection Agency-Mumbai, PTH
&
T Maheswara Rao
Technical Officer
Export Inspection Agency-Mumbai, PTH
Introduction

To ensure food safety and quality, food samples require certain microbiological tests considering following relevant points -

- The homogeneity of the food, the relative sizes of the sample to be taken.
- The potential degree of variation of the parameters for analysis,
- The significance and intended use of the analytical result.
Entry point

• Change room mandatory requirement
• In the change room all **Personal Protective Equipments (PPEs)** must be available which are lab coat, mask, cap, shoe cover / slipper and gloves.
• Suitable Hand disinfectant
• Restricted entry to avoid chances of contamination
Sample receiving

• The sample should be protected against extraneous contamination.

• **The temperature** at the time of collection and upon receipt is useful to the laboratory for the interpretation of results.

• A sample container should not be more than three-quarters full in order to avoid leakage and to allow proper mixing of the sample in the laboratory.

• Identify samples clearly and completely, and record the sample information in log book.
Media Preparation

• **Distilled water** should be used

  [Minimum Acceptance criteria conductivity 25 $\mu$Scm$^{-1}$, TPC <100 CFU/mL, free of any inhibitory agent]

• **Post sterilization pH check mandatory.** pH paper not recommended.

• pH calibration daily with three buffer-4.0;7.0;9.0

• **Log book** - date of preparation, media name, lot no, wt.& qty, mode of sterilization, measured pH, prepared by and verified by.

• Balance for only media preparation; can not be used for sample preparation

• Daily checks of balance with single weight.
Media Preparation

• Media used in pour plate methods, which are added to the sample, are tempered to 44 °C to 47 °C, using a water bath set at 44 °C to 47 °C.

• Heat-labile supplements should be added to the medium after it has been cooled to below 50 °C.

• Pour the molten agar culture medium into Petri dishes so as to obtain a thickness of at least 3 mm (e.g. for 90 mm diameter dishes, 18 ml to 20 ml of agar are normally required).

• For surface inoculation of solid culture media, dry the plates shortly before use until the droplets have disappeared from the surface of the medium. Do not over-dry the plates.
Sterilization

• Autoclave – Used for sterilization of media/Buffer etc. **Same can not be used for disposal.**
• 121± 3⁰C for 15 min
• Performance Verification –
  Chemical strip every batch/cycle
  Biological strip every month
  External Calibration against temperature
  Use of timer recommended.
  Pressure gauge calibration not mandatory.
Method, equipment & analysis

• **Use of latest national/international std. method**
• Analysis must be performed by a qualified, trained & competent analyst.
• Examine the samples as soon as possible after receipt, preferably within 24 h (perishable), or as agreed with the parties concerned.
• Pan balance for sample weighing; daily checks with single weight; the maximum permissible errors should be 1% or better when weighing out test samples & record in log book.
• The sample container should be wiped with disinfectant and opened for just long enough to allow the sample to be transferred and closed immediately afterwards.
• **Test should be performed in a separate closed area under LAF or near to burner flame (50 cm dia).**
Method, equipment & analysis

• Sample homogenization: Use of stomacher / bag mixer preferred. Usual operating time 1 min to 3 min.

• **During serial dilution mouth pipetting prohibited**; Use of micropipette recommended.

• Incubator used should be of 0.1 resolution with temperature display. Observation twice daily by working thermometer or by data logging system.

• More than six plates should not be stacked while keeping for incubation in the incubator.

• Use of anaerobic system where necessary
Method, equipment & analysis

• **Log book** - Date, sample no, temperature, time in & time out, name of analyst & verifier
• Use of reference culture as **positive control**
• Use of **negative control** (buffer control, plate control, area control) wherever applicable during analysis
• Should consider both **typical & atypical colony** for pathogen detection
• Lab must have adequate biochemical reagents & antisera for carrying out confirmatory test
• Should use proper formula for enumeration of quantitative parameters like – TPC
• Should observe & record the result
• Proper use of units according to parameters in the test report
Method of calculation for TPC

- Calculate the number $N$ of microorganisms present in the test sample as a weighted mean from two successive dilutions using Equation

$$N = \frac{\Sigma C}{V \times 1.1^d}$$

where

- $C$ is the sum of the colonies counted on the two dishes retained from two successive dilutions, at least one of which contains a minimum of 10 colonies;
- $V$ is the volume of inoculum placed in each dish, in millilitres;
- $d$ is the dilution corresponding to the first dilution retained [$d = 1$ when the undiluted liquid product is retained]
Example

Counting has produced the following results:
at the first dilution retained (10–2): 168 cfu
at the second dilution retained (10–3): 14 cfu

\[ N = \frac{\Sigma C}{Vx1.1^xd} = \frac{168 + 14}{1 \times 1.1 \times 10^{-2}} = \frac{182}{0.011} = 16545 \]

Rounding off the result as specified above, the number of microorganisms is 17 000 or 1,7 \(10^4\) per gram of product.
Method of calculation: after identification

When the method used requires identification, a given number $A$ (generally 5) of presumptive colonies is identified from each of the dishes retained for the colony counting. After identification, calculate, for each of the dishes, the number $a$ of colonies complying with identification criteria, using Equation:

$$a = \frac{b}{A} \times C$$

where

- $b$ is the number of colonies complying with identification criteria among the identified colonies $A$;
- $C$ is the total number of presumptive colonies counted on the dish.
Safety cabinet

**Horizontal laminar airflow cabinets** protect the *product* from contamination, but blow any aerosols generated into the operator’s face.

**Vertical laminar airflow cabinets** protect the *product* by the use of vertical laminar flow of HEPA-filtered air. They also protect the *operator* by the use of internally recirculated air.

**Class II safety cabinets** protect the *product*, *the operator and the environment*. They recirculate some filtered air, exhaust some to the atmosphere and take in replacement air through the working aperture, thereby providing operator protection. **They are suitable for work with risk category 3 pathogens.**
Method, equipment & analysis

- **Refrigerator** – For the conservation of perishable food samples for analysis, the temperature shall be 3 °C ± 2 °C. For other uses, the temperature, shall be 5 °C ± 3 °C.

- **Deep Freezer** – The temperature, shall be below −15 °C, preferably below −18 °C for highly perishable food samples.

- **Ultra low Deep Freezer** – Reference stock cultures are maintained at −70 °C or below.
Use of reference culture

The use of reference culture is mandatory for –

- Quality control of microbiological test, media verification & kit verification
- Verification of test method before use
- To compare between microbiological techniques

The laboratory must use reference culture from a certified/reputed culture collection centre (MTCC, ATCC or ATCC derivative etc) for traceability
Handling & storage of Reference culture

Reference Culture
- Cultured once
- Check purity

Reference stock
(long term storage at – 70°C & below)
- Thaw/reconstitute – cultured once

Working stock
(Short term storage at 4°C)
- Check purity

Working culture
Disposal

• **Separate autoclave** for disposal of used media/tubes/flasks etc.

• **At 121±3°C for 30 min**

• **Log book** - date; items kept; temperature & time of retention; sticking of used chemical strips; performed by & verified by

• **Discard the sterilized material through authorized biomedical waste collection organization.**
Quality Control

✓ Use of positive & negative control during test
✓ Spike study
✓ Reproducibility
✓ Participation in ILC/PT program

Set the evaluation criteria
Use of alternative method

If the food business operator/laboratory wishes to use analytical methods other than std. national/International methods, the methods shall be validated according to internationally accepted protocols and their use authorised by the competent authority.

Use of alternative method

The food industry needs to release safe products in as little time as possible:

- Product demands
- Short shelf life products
- Storage cost
- Give solution to problems: fast decision making
- Fast diagnosis of control plans.
- Fast answer in a food crisis: emerging pathogens
## Use of alternative method

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<th>Disadvantage</th>
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<td>• Speed in results</td>
<td>• Cost of equipment and reagents</td>
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<td>• Reduction in laboratory labouring</td>
<td>• Limitations in application</td>
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<tr>
<td>• Higher productivity</td>
<td>• Acceptance by clients/Regulatory authority</td>
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<td>• Easier interpretation of results</td>
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Conclusion

- The principle objective of a laboratory is to produce reliable result which is therefore the activity to which due attention must be paid.
- The general objective of the laboratory might be defined as producing analytical data of adequate accuracy and reliability within an acceptable time.
Thank you