

**SAIPRO INDUSTRIES PVT. LTD.**

**TOTAL YEAST AND MOULD COUNT FOR FINISHED GOODS AND RAW MATERIALS**

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| **SOP NO** | **VERSION NO** | **REVISION DATE** |
| SI/SOP/QC/34 | 00 | - |
| **ISSIUE DATE** | **EFFECTIVE DATE** | **REVIEW DATE** |
| 1st Oct 2022 | 1st Nov 2022 | 1st Nov 2024 |
| **PREPARED BY** | **CHECKED BY** | **APPROVED BY** | **AUTHORIZED BY** |
| QA OFFICER | QA EXECUTIVE | QA MANAGER | QA HEAD |
|  |  |  |  |

**OBJECTIVE**

 To lay down a procedure for Total Yeast and mould count of finished products to determine microbial load.

**SCOPE**

 The SOP shall be applicable for enumeration of yeast and mould count.

 **RESPONSIBILITY**

* Preparation: Officer and above
* Checking: Executive and above
* Approval: Department Head and above
* Authorization: QA Head

 **ACCOUNTABILITY**

 Head of the Concerned Department

# **Media:**

# Chloramphenicol yeast glucose agar

* Buffered sodium chloride peptone solution

**Procedure:**

**Sample preparation:**

* Make a 1:10 dilution of the well mixed sample, by aseptically transferring sample to the desired volume of diluent.
* Measure non-viscous liquid samples (i.e.Viscosity not greater than milk) volumetrically and mix thoroughly with the appropriate volume of diluent (11 ml into 99 ml, or 10 ml into 90 ml or 50ml into 450 ml).
* Weigh viscous liquid sample and mix thoroughly with the appropriate volume of diluent (11 + 0.1g into 99ml; 10+ 0.1g into 90ml or 50+0.1g into 450ml).
* Weigh 50+0.1g of solid or semi-solid sample into a sterile blender jar or into a stomacher bag. Add 450 ml of diluent. Blend for 2 minutes at low speed (approximately 8000 rpm) or mix in the stomacher for 30-60 seconds.
* Powdered samples may be weighed and directly mixed with the diluent. Shake vigorously.
* In most of the food samples particulate matter floats in the dilution water. In such cases allow the particles to settle for two to three minutes and then draw the diluent from that portion of dilution where food particles are minimum and proceed.

# **Pour plating:**

* Label all petri plates with the sample number, dilution, date and any other described information.
* Pipette 1ml of the food homogenate of such dilutions which have been selected for plating into a petri dish in duplicate.
* Pour 10-12 ml of the agar medium (tempered to 45oC). Mix by swirling and allow to solidify.

 (OR)

* Add 2ml antibiotic solution to 100ml of plate count, malt agar. Mix and pour 10-12ml of the agar medium tempered to 45oC. Mix by swirling and allow to solidify.

4.**Incubation:**

* Invert plates and incubate at 20 or 25oC for 5 days. Discard plates after five days if growth is not observed, observe plates every day and mark the colonies because some time fungal growth spreads to entire plate and mask the colonies. Do not open the plates which are showing fungal sporangia.

# **Counting colonies:**

 Count colonies, multiply by the inverse of the corresponding dilution and report as yeast or mould count per g or ml.

1. **Reporting:**

Yeast and Mould count =

1. **REFERENCES**
* Compendium of methods for the microbiological examination of foods.(1992) Carl Vanderzant and Don F. Splittstoesser. Eds. Washington D.C.p.239 – 249.
1. **ABBREVIATIONS**
* ml - millilitre
* g – gram
* CFU – Colony forming unit
1. **ATTACHMENTS**

 **TEST DATA SHEET FOR TOTAL YEAST AND MOULD COUNT**

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| --- | --- | --- | --- |
| **FORMAT NO** | **VERSION NO** | **EFFECTIVE DATE** | **NEXT REVIEW DATE** |
| SIPL/SOP/QC/34/ F-11 | NIL | 1ST Nov 2022 | 1st Nov 2024 |

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| --- |
| **PRODUCT NAME:**  |
| **BATCH CODE:** |
| **MFG date:**  | **EXP date:**  |
| **DATE OF ANALYSIS:** | **DATE OF RESULT:** |

# **Media used:**

* **Incubator ID/ Temperature:**
* **Incubation started:**
* **Incubation end:**
1. **TOTAL YEAST COUNT:**
2. **Counting colonies:**
* Count colonies, multiply by the inverse of the corresponding dilution and report as yeast or mould count per g or ml.
* **Observation:**
* Note down the observation for negative control (plate should have no growth): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* If growth is observed in negative control repeat the experiment\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Count the number of colonies appeared on the plate on incubation for **3rd day (72hours)** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Count the number of colonies appeared on the plate on incubation for **4th day (96hours)** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* Count the number of colonies appeared on the plate on incubation for **5th day (120hours)** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
* **Calculation**

∑ C

 N=

(N1+0.1N2) D

* ∑ C =the sum of the colonies counted on all the plates
* N 1 = the number of plates counted in the first dilution.
* N 2 = the number of plates counted in the second dilution.
* D = the dilution from which the first counts were obtained (for example, 10-1).

**Calculation:**

1. **Reporting:**

Yeast and Mould count =

**Done by Checked by**

\*END OF SOP\*