# PROTOCOL
# FOR
# HOLD TIME VALIDATION FOR CULTURE SUSPENSION

# (10-100 cfu/ 0.1 ml)

# Culture Suspension Validation

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# Culture Suspension Validation

## 1.0 PROTOCOL APPROVAL

This document is prepared by the Microbiology Department for hold time of 10-100 CFU/0.1 ml culture suspension.

| PREPARED BY | | |
|---|---|---|
| **NAME/ FUNCTIONAL AREA** | **DESIGNATION** | **SIGNATURE /DATE** |
| | | |
| **MICROBIOLOGY** | | |

| CHECKED BY | | |
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| **NAME/ FUNCTIONAL AREA** | **DESIGNATION** | **SIGNATURE /DATE** |
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| | | |
| **QUALITY CONTROL-HEAD** | | |

| APPROVED BY | | |
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| **NAME/ FUNCTIONAL AREA** | **DESIGNATION** | **SIGNATURE /DATE** |
| | | |
| **VALIDATION- QUALITY ASSURANCE** | | |
| | | |
| **QUALITY ASSURANCE-HEAD** | | |

# Culture Suspension Validation

## 2.0 OBJECTIVE

This protocol is designed to validate the documented evidence that to ensure hold time period of culture suspension in 0.9% normal saline solution, used for growth promotion test & other microbiology related validation i.e. sterility method validation, disinfectant validation etc.

## 3.0 SCOPE

The scope of this protocol shall be limited to validate hold time period of culture suspension in 0.9% normal saline solution and no impact on viability of cultures, when stored at 2-8°C in 0.9% Normal Saline Solution.

## 4.0 RESPONSIBILITY

Responsibility of different Department/Personnel involved in different activities related to the valuation study.

| Functions | Responsibility |
|---|---|
| Microbiology | Preparation of protocol & report |
| Microbiology | Review of the protocol & Execution |
| Quality assurance | Review & Approval of protocol & report |
| Quality assurance | Approval of the executed protocol and report |

## 5.0 REFERENCE DOCUMENT

Following documents are referred during preparation of the protocol:

| Document Name | Document Number |
|---|---|
| Working culture dilution preparation | |

## 6.0 METHODOLOGY

### 6.1 Pre-Requisites

#### 6.1.1 Culture Organisms to be used -

a) *Staphylococcus aureus* ATCC 6538
*b)* *Pseudomonas aeruginosa* ATCC 9027
*c)* *Clostridium sporogenes* ATCC 11437
*d)* *Bacillus spizizenii* ATCC 6633
*e)* *Candida albicans* ATCC 10231
*f)* *Aspergillus brasiliensis* ATCC 16404
*g)* *Escherichia coli* ATCC 8739
*h)* *Salmonella spp.* NCTC 6017
*i)* *Environmental* isolate - I

*j)* *Environmental* isolate – II
*k)* *Environmental* isolate – III

6.1.2 **Accessories and Media:** LAF, Micropipette, Sterile tips, BOD incubator, SCDA, SDA/SCA and RCA media plates.

6.1.3 **Preparation of 10 - 100 CFU / 0.1 ml -** Prepare the 10 - 100 CFU / 0.1ml suspension of culture organisms as per the SOP (working culture dilution preparation).

6.2 **Validation Procedure:**

6.3.1 Microbiologist shall prepare the 10-100 cfu/0.1 ml culture suspension as per SOP "Working Culture Dilution Preparation".

6.3.2 Store the dilutions at 2-8°C in Cooling Incubator.

6.3.3 Take out the quantified dilution (Contains 10-100 cfu /0.1ml) of each culture suspension from the Cooling Incubator 30 minutes prior to the testing and transfer it to LAF so as to acclimatize with the working environment.

6.3.4 Take 0.1ml of test suspension of specific test organism (10-100 cfu) in duplicate sterile petri-plates and pour SCDA, SDA/SCA and RCA media (cooled to around 45ºC).

6.3.5 Rotate the plates in clockwise and anticlockwise directions for homogenous mixing and let the media solidify.

6.3.6 Incubate the plates at 30-35ºC for 3 days for bacterial culture and 20-25ºC for 5 days for fungal cultures.

6.3.7 Count the number of colonies of both duplicate plate and record it as Avg. CFU/0.1ml.

6.3.8 Round off to complete number wherever average counts observed is in decimal places. For e.g., if the average count is 32.5 then report the result as 33 CFU/0.1ml.

6.3.9 This shall be considered as 'First day' Results.

6.3.10 Perform the testing of stored Culture suspension daily up to 10 days.

6.3.11 After testing Store the dilutions at 2-8°C in Cooling Incubator.

6.3.12 Incubate one plate of all agar media as a negative control along with test plate.

6.3.13 The results shall be observed and recorded as per respective observation sheet.

**7.0 VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA**

7.1 In the Negative control no colonies should be observed.

7.2 There should be no impact on viability of cultures.

7.3 Observed count should not be less than 70 % of Initial quantified culture up to the holding period.

7.4 **Revalidation**

7.4.1 Revalidation shall be done whenever the storage condition changed.

**8.0 SUMMARY OF VALIDATION REPORT:**

The validation report shall consist of a summary document, in the narrative form, which briefly describes the work as well as conditions regarding acceptability. This validation report shall also include the raw data, which shall be completed at the time of validation activity as per annexure.

## 9.0 APPROVAL OF VALIDATION REPORT:

All validation parameters should comply with acceptance criteria as per protocol, reviewed and signed by Quality assurance.

## 10.0 ANNEXURE:

Following documents (Annexure) are enclosed as a part of protocol and shall be pre-approved as a part of main protocol.

| S.No. | Document | Title | Data sheet Number |
|---|---|---|---|
| 1. | Annexure I | Observation Sheet for Hold Time of Culture Suspension (10-100 cfu/0.1ml) | |

## 11.0 ABBREVIATIONS:

| Abbreviation | Terms |
|---|---|
| SCDA | Soyabean Casein Digest Agar |
| CFU | Colony Forming Unit. |
| SDA | Sabouraud Dextrose agar |
| SCA | Sabouraud Chloramphenicol agar |
| RCA | Reinforced Clostridia Agar |
| LAF | Laminar Air Flow |
| % | Percentage |
| ml | Milliliter |
| $^0$C | Degree Celsius |

## 12.0 CHANGE HISTORY DETAILS:

| Version no. | Reason for revision | CRF no. | Effective date |
|---|---|---|---|
| 00 | First Issue | Not Applicable | |

## Culture Suspension Validation

**ANNEXURE I**

**Observation Sheet for Hold Time of Culture Suspension (10-100 cfu/0.1ml)**

**Date of Testing:** **Date of Report:**

**Media Detail:**

| | | | |
|---|---|---|---|
| Name of Media | | | |
| Sterile Media Lot No. | | | |
| Incubation Temp. | | | |
| Incubation Time | | | |
| Incubator ID No. | | | |
| Negative Control | | | |
| Analyzed by | | | |

**Observation:**

| Name of Culture | Culture Ref. No. | Initial count (cfu/0.1ml) | Observations(cfu/0.1ml) | | | % Recovery |
|---|---|---|---|---|---|---|
| | | | Plate-I | Plate-II | Avg. | |
| *Staphylococcus aureus* | | | | | | |
| *Pseudomonas aeruginosa* | | | | | | |
| *Clostridium sporogenes* | | | | | | |
| *Bacillus spizizenii* | | | | | | |
| *Candida albicans* | | | | | | |
| *Aspergillus brasiliensis* | | | | | | |
| *Escherichia coli* | | | | | | |
| *Salmonella spp.* | | | | | | |
| Environmental isolate - I (……...) | | | | | | |
| Environmental isolate – II (……...) | | | | | | |
| Environmental isolate – III (……...) | | | | | | |

**Remark:**

Observed by/ date: Reviewed by/ date: