PROTOCOL No.		**Hold Time Validation for Sterilized Media (Prepared Media Plates and Tubes)**
SUPERSEDES	**NIL**	
EFFECTIVE DATE		
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PROTOCOL

FOR

HOLD TIME VALIDATION FOR

STERILIZED MEDIA

(PREPARED MEDIA PLATES AND TUBES)

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1.0 APPROVAL SIGNATURE:

This is specific protocol for Hold Time Validation for Sterilized media. The Author signature indicates that this document has been prepared in accordance with existing standards and adequately reflects the tasks and deliverables necessary for qualification.

Prepared by/Functional area	Designation	Signature	Date
Quality Control-Microbiology			

The reviewer's signature indicates that, this document has been reviewed and it accurately and completely reflects the tasks and deliverables necessary for process.

Checked by/Functional area	Designation	Signature	Date
Quality Control-Microbiology			
Quality Control			
Validation – QA			

The approver's signature indicates that the documentation and information contained herein complies with applicable regulatory, corporate, divisional/departmental requirements, and current Good Manufacturing Practices.

Approved by/Functional area	Designation	Signature	Date
Quality Assurance			

2.0 OVERVIEW:

2.1 Objective:

The objective of this protocol is to ensure hold time period of Sterilized media used for routine Microbiological testing and Environmental Monitoring.

2.2 Purpose & Scope:

The purpose is to provide high degree of assurance that the sterilized Media is free from any type of Microbial contamination & there is no change in physical and biological activity during hold time.

2.3 Responsibility:

To conduct the qualification study, a team shall be formed. The team shall contain the members from the, Quality Control and Quality Assurance departments. The Validation team is described through the following responsibility.

Quality Control (Microbiology)	To prepare the protocol
	To provide the training
	To conduct the qualification study
Quality Control and Quality Assurance	To review the protocol
	To assure the adequate functioning of system
QA Head	To approve validation protocol
	To approve validation report

3.0 EXECUTION TEAM:

Following personnel shall be responsible for the execution of qualification study;

Executive – QC (Microbiology) : To conduct the qualification study as per protocol.

Executive Quality Assurance : To review & monitor the qualification study.

4.0 TRAINING RECORD:

4.1 Purpose:

The purpose of the training is to familiarize the trainees with the requirement of Hold Time Validation Study.

4.2 Scope:

This Training is applicable to the all concerned persons which are involved in this activity.

4.3 Topics:

The following topics shall be covered during training:

4.3.1 Purpose & procedure of Hold Time Validation study.
4.3.2 Identifying the responsibility of involved person.
4.3.3 Documentation practices to be followed.
4.3.4 General precautions / guidelines to be followed during qualification.
4.3.5 Attach training record with the report as **Annexure –**.

5.0 REQUIREMENTS FOR QUALIFICATION:

5.1 Equipment:

5.1.1 Biosafety Cabinet
5.1.2 Autoclave (HPHV Steam Sterilizer)
5.1.3 Autoclave (Vertical Autoclave)
5.1.4 Colony Counter
5.1.5 Balance
5.1.6 pH Meter
5.1.7 Incubator (20^0C to 25^0C)
5.1.8 Incubator (30^0C to 35^0C)
5.1.9 Laminar Air Flow Bench
5.1.10 Micropipette

5.2 Material:

5.2.1 Sterilized Media
5.2.2 Sterile Petri Plates
5.2.3 Test tubes
5.2.4 Spreader
5.2.5 General glassware

5.2.6 Sterilization indicator tape

5.2.7 Micropipette tips

6.0 QUALIFICATION PROCEDURE OR METHODOLOGY:

6.1 General Recording Instructions:

6.1.1 Read the contents of the document thoroughly before proceeding for Execution of the activity (in case of doubts/contradictions/contact the approvers of the document for clarifications).

6.1.2 Recording of all the observations and data shall be as per good documentation practices

6.3 Validation Methodology:

6.3.1 The study will be conducted to validate the hold time period upto which the media maintain its nutritive properties and can be utilized for analysis purpose and after which the media shall not be used in any of the test and should be discarded, when stored at specified temperature (20 to 25^{0}C). These parameters i.e. physical description, pH, GPT and weight (for prepared media plates) shall be performed on all microbiological media mention in table 01.

TABLE 01: Parameters studied were:

3 batches of each media shall be evaluated for following parameters.

S.No.	MEDIA	PARAMENTER	INTERVAL (in days)
1.	Soyabean Casein Digest Medium (SCDM)	Physical description	2, 7, 14, 21 & 28
		pH (7.3 ± 0.2)	2, 7, 14, 21 & 28
		GPT	2, 7, 14, 21 & 28
2.	Fluid thio-glycollate Medium (FTM)	Physical description	2, 7, 14, 21 & 28
		pH (7.1 ± 0.2)	2, 7, 14, 21 & 28
		GPT	2, 7, 14, 21 & 28
3.	Soyabean-Casein Digest Agar (SCDA)	Physical description	2, 3, 5, 7 & 10
		pH (7.3 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
4.	Sabouraud Chloramphenicol Agar (SCA)	Physical description	2, 3, 5, 7 & 10
		pH (5.6 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
5.	Sabouraud Dextrose Agar (SDA)	Physical description	2, 3, 5, 7 & 10
		pH (5.6 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10

S.No.	MEDIA	PARAMENTER	INTERVAL (in days)
		Weight	Daily
6.	Cetrimide Agar (CA)	Physical description	2, 3, 5, 7 & 10
		pH (7.2 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
7.	Mannitol-Salt Agar (MSA)	Physical description	2, 3, 5, 7 & 10
		pH (7.4 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
8.	Plate Count Agar (PCA)	Physical description	2, 3, 5, 7 & 10
		pH (7.0 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
9.	R2A	Physical description	2, 3, 5, 7 & 10
		pH (7.2 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
10.	MacConkey Broth (MB)	Physical description	2, 3, 5, 7 & 10
		pH (7.3 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
11.	Mac Conkey Agar (MA)	Physical description	2, 3, 5, 7 & 10
		pH (7.1 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
12.	Xylose-Lysine-Deoxycholate Agar Medium (XLDA)	Physical description	2, 3, 5, 7 & 10
		pH (7.4 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
13.	Reinforced Clostridial Agar (RCA)	Physical description	2, 3, 5, 7 & 10
		pH (6.8 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
14.	E.E. BROTH (EEB)	Physical description	2, 3, 5, 7 & 10
		pH (7.2 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
15.	Violet Red Bile Glucose Agar (VRBGA)	Physical description	2, 3, 5, 7 & 10
		pH (7.4 ± 0.2)	2, 3, 5, 7 & 10
		GPT	2, 3, 5, 7 & 10
		Weight	Daily
16.	Rappaport Vassiliadis Salmonella Enrichment	Physical description	2, 3, 5, 7 & 10
		pH (5.2 ± 0.2)	2, 3, 5, 7 & 10

S.No.	MEDIA	PARAMENTER	INTERVAL (in days)
	Broth (RVSEB)	GPT	2, 3, 5, 7 & 10

6.3.2 Culture suspension used for growth promotion test shall be prepared and qualified as per current version SOP -"Preparation and usage of 10-100 cfu's/mL culture suspension" for containing 10-100 cfu/mL employing standard pour plate technique. The microorganism to be used in the hold time study are given below:

TABLE 02

S.No.	Media	Media Used to Test	Test Microbial culture
1.	Soyabean-Casein Digest Agar (SCDA)	Environmental monitoring, sub culturing & culture suspension	*S. aureus* (S.a.)
			B.subtilis (B.s.)
			P.aeruginosa (Ps.a)
			E.coli (E.c.)
			C. albicans (C.a.)
			A. niger (A.n.)
2.	Soyabean-Casein Digest Medium (SCDM) (Other than sterility test)	MLT, Water Testing BI Inoculation	*S. aureus* (S.a.)
			B.subtilis (B.s.)
			P.aeruginosa (Ps.a)
			E.coli (E.c.)
			S. abony (Sl.a)
3.	Soyabean-Casein Digest Medium (for sterility test)	Sterility	*B.subtilis* (B.s.)
			C. albicans (C.a.)
			A. niger (A.n.)
4.	Sabouraud Chloramphenicol Agar (SCA)	Environmental monitoring, sub culturing & culture suspension	*C. albicans* (C.a.)
			A. niger (A.n.)
5.	Sabouraud Dextrose Agar (SDA)	Environmental monitoring	*C. albicans* (C.a.)
			A. niger (A.n.)
6.	Cetrimide Agar (CA)	MLT, Water Testing	*P.aeruginosa* (Ps.a)
			E.coli (E.c.)
7.	Mannitol-Salt Agar (MSA)	MLT, Water Testing	*S. aureus* (S.a.)
			E.coli (E.c.)
8.	Plate Count Agar (PCA)	Water Testing	*P.aeruginosa* (Ps.a)
			S. aureus (S.a.)
			E.coli (E.c.)
			S. abony (Sl.a)
9.	R_2A	Water Testing	*P.aeruginosa* (Ps.a)
			S. aureus (S.a.)
			E.coli (E.c.)

S.No.	Media	Media Used to Test	Test Microbial culture
10.	MacConkey Broth (MB)	MLT, Water Testing	*S. abony* (Sl.a)
			E.coli (E.c.)
			P.aeruginosa (Ps.a)
11.	MacConkey Agar (MA)	MLT, Water Testing	*E.coli* (E.c.)
12.	Xylose-Lysine-Deoxycholate Agar Medium (XLDA)	MLT, Water Testing	*S. abony* (Sl.a)
			E.coli (E.c.)
13.	Reinforced Clostridial Agar (RCA)	sub culturing & culture suspension	*Cl.sporogenes* (Cl.s.)
14.	Fluid thio-glycollate Medium (FTM)	Sterility	*Cl.sporogenes* (Cl.s.)
			P. aeruginosa (Ps.a)
			S. aureus (S.a.)
15.	E.E. BROTH (EEB)	MLT, Water Testing	*E.coli* (E.c.)
			P.aeruginosa (Ps.a)
16.	Violet Red Bile Glucose Agar (VRBGA)	MLT, Water Testing	*E.coli* (E.c.)
			P.aeruginosa (Ps.a)
17.	Rappaport Vassiliadis Salmonella Enrichment Broth (RVSEB)	MLT, Water Testing	*S. abony* (Sl.a)
			S. aureus (S.a.)

6.3.3 Preparation of media:

6.3.3.1 Prepare the 3 different lot of each media (mention in table 01) as per the vendor's instructions given on the box or leaflet.

6.3.3.2 Take approximately 1/4th or 1/2 of the required quantity of purified water in the flask/bottle in case of Agar media & WFI in case of Broth media.

6.3.3.3 Weigh the media as per the quantity mentioned on the media box and take the print out as per current version of SOP "Operation, Calibration, Cleaning and Maintenance of Electronic Weighing Balance"

6.3.3.4 Mix the media in the given purified water/WFI. Heat the solution if necessary in order to dissolve the media.

6.3.3.5 Make up the Volume as per the required quantity to be prepared.

6.3.3.6 Check the pH of media as per current version of SOP "Operation, Cleaning, Calibration & Maintenance of pH meter" make entry in pH log book.

6.3.3.7 If pH before autoclaving is out of limit then adjust with 0.1N NaOH or 0.1N HCl.

6.3.3.8 Note all the details in **Format** 'Media Preparation Record'.

6.3.3.9 Every prepared media will be allocated with an internal media lot number i.e. "xxxx-yy". Where "xxxx" is the serial number of the media prepared & "yy" stands for last two digit of calendar year.

6.3.3.10 Sterilize the media as per the validated cycle & follow the Current version of SOP "Operation, Cleaning, calibration and maintenance of HPHV Steam Sterilizer" for operation of Autoclave.

6.3.3.11 After sterilization, unload the media and allow the liquid media to cool to room temperature. Pour the agar media into the respective media plates, once the temperature has reached 45°C.

6.3.3.12 After the liquid media has reached to room temperature or agar plates are solidified, take the pH once again & record it in the respective log books.

6.3.3.13 Prepared media plates and tubes should be pre incubated for 24- 48 hrs at 30 to 35^0C temperature.

6.3.3.14 After completion of preincubation, check the plates/tubes for microbial growth or any contamination. If contaminated plates found, remove those plates/tubes.

6.3.3.15 Store the remaining media plates/tubes at 20 to 25^0C temperature for 10 days except SCDM and FTM tubes for 28 days.

6.3.3.16 Remove the required quantity of media plates/tubes from the plates/tubes under storage as per specified time interval define in table 01 and observe the physical description of the media, pH, weight (for media plates) and perform growth promotion test. Record the results as per annexures.

6.3.4 Physical Description of the Media:

6.3.4.1 For Solid agar media, observe the plates for cracks, dehydration, shrinkage, dimpled surfaces etc.

6.3.4.2 For liquid media, observe the tubes for any visible growth and check the colour of the solution and results are recorded as per annexure 00.

6.3.5 Weight:

6.3.5.1 Weight of 10 plates for 3 different lot of every agar media was taken daily and results were expressed in grams for 10 days and results are recorded as per annexure 00

6.3.6 pH:

6.3.6.1 Take the 5 media plate/tube for 3 different lot of every media and observe the pH as per current version of SOP "Operation, Cleaning, Calibration & Maintenance of pH meter" and result are recorded as per annexure 00

6.3.7 Growth Promotion Test:

6.3.7.1 Take the media plates/tubes for 3 different lot of every media and perform the growth promotion test as per current version SOP "Preparation and Qualification of sterile media" Result are recorded as per annexure 05.

6.3.7.2 A concurrent viable count should be carried out when performing GPT, as a check that the working dilution has been correctly prepared and calculated.

6.3.7.3 Spread out the standard culture in freshly prepared SCDA/RCA medium for standard bacterial cultures and on SCA plate for standard fungal cultures to get concurrent viable counts as positive control.

6.3.7.4 Incubate all the bacterial culture for 2 to 3 days at 32ºC± 2.5ºC and all fungal cultures at 22.5ºC±2.5ºC for 3 to 5 days.

6.3.7.5 Observe the media tubes/plates daily (until the end of incubation) and record the observations as per annexure 00.

7.0 ACCEPTANCE CRITERIA:

7.1 The colour of the media should not change throughout the storage period.

7.2 pH should be within the limits throughout the storage period.

7.3 Media used for bacterial growth should be show visible growth with in 48 hrs at 32ºC± 2.5ºC temperature.

7.4 Media used for fungal growth should be show visible growth with in 120 hrs at 22ºC± 2.5ºC temperature.

7.5 Compare the weight loss from 1st day to 10th day. It should be NMT 10 % of the initial weight.

7.6 Concurrent viable count of the standard microorganisms shows more than 70 % recovery of the initial count.

7.7 The media under test should show more than 70 % recovery in comparison to the concurrent count.

7.8 Negative controls do not show any growth of the microorganisms for the incubation period.

8.0 REVALIDATION SCHEDULE: Revalidation shall be carried out in case of:

8.1 Whenever a change in media composition or new Vendor.

8.2 In case of sterilization parameters changed.

8.3 Whenever the storage condition changed.

9.0 OBSERVED DEVIATION (IF ANY):

The Deviation / Discrepancy shall be addressed as per the **SOP**".

10.0 QUALIFICATION REPORT:

The qualification report shall consist of a summary document, in narrative form, which shall briefly describe the activity performed along with the observations recorded in relevant exhibits.
This report shall also include the related documents and attachments / exhibit which were completed at the time of qualification activity.

11.0 ABBREVIATIONS:

11.1 Nil

12.0 LIST OF ANNEXURES:

Annexure No.	Annexure Title
	Training Record
	Report for Physical description of the media
	Report for Weight (for Prepared media Plates)
	Report for pH of the media
	Report for Growth Promotion Test of the media

13.0 REFERENCE DOCUMENT:

Nil