PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE	
Department: Microbiology	**SOP No.:**
Title: Investigation of Out of Specification of Test Results in Microbiology	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

The purpose of this SOP is to establish a procedure for the routine handling of out of specification (OOS) microbiology laboratory results. The investigation or failure investigation should whenever possible identify the cause of the OOS and evaluate its impact.

2.0 SCOPE:

This SOP is applicable to OOS test results of / Total aerobic microbial count and test for specified microorganism are obtained from Raw Material, In-process stage, Finished Products and Stability samples or during OOS results obtained from Water samples / Environmental monitoring / Compressed air / Nitrogen gas, Growth Promotion Test in microbiology section.

3.0 RESPONSIBILITY:

Officer/Executive – Quality Control (Microbiology)

Head - Quality Control

Head- Production

Head - Quality Assurance

4.0 PROCEDURE:

In case of out of specification test result observed, analyst shall be inform to head microbiology immediately and head microbiology shall inform to head QC immediately.

Identification and Assessing of Out of specification and followed by its investigation shall be done as given in OOS flow chart diagram as mentioned in Annexure – II.

Head QC or designee shall inform to Head-QA. QA person shall record the details in the “OOS Investigation Form Issuance Register” (Annexure-I) and issue the control copy of respective OOS investigation form.

A unique number shall be allotted to the form. All forms shall be numbered in the form **OOSM/ XXX / YY**, where

OOSM - stands for Out of specification for microbiology,

/ - stands for forward slash

XXX - stands for Sequential serial number starting from 001

/- stands for forward slash

YY- stands for Current year e.g. 22 for 2022,etc.

(e.g. First OOS Investigation form in the year 2022 shall be given form no. as OOSM/001/22)

4.1 Failure investigation of Water:

4.1.1 Water Failure investigation shall be carried out as described in the flow chart diagram as per Annexure-II.

4.1.2 If the counts exceeds to alert or action limit in any type of water, analyst shall be inform to head microbiology immediately. Head microbiology shall inform to Head Quality control and Head QA and to the concerned department as per alert action intimation form of SOP: Sampling & analysis of water.

4.1.3 The failure water results are indicated by the development of growth on solid agar surface and turbidity in the liquid.

4.1.4 Identify the colony characteristics of the bacteria / fungi observed and carry out identification as per SOP: Procedure for identification of microbial cultures/microbial isolates .if the test is showing the presence of pathogens, confirm it with confirmatory test.

4.1.5 Inform QA department to quarantine the batches manufactured during the water failure period. QA should initiate the full scale investigation to find out the exact cause of failure if observed results more than action limit.

4.1.6 Laboratory Investigation shall be carried out as per Annexure– III but not limited to the parameter identified.

4.1.7 Investigation shall be extended to previous and subsequent A R Number to find out the root cause and to prevent reoccurrence.

4.1.8 If assignable cause identified during laboratory investigation, and if it is corrected only through calculation then correct the same and report the results.

4.1.9 Assignable cause identified in laboratory investigation and if analysis is required to arrive at conclusion then QC Head, QA Head collectively decided to extent of the sampling and evaluates

the impact / risk.

4.1.10 If no assignable cause identified during laboratory investigation, for further investigation hand over to QA.

4.1.11 QA will investigate cross functional investigation for identification route cause as per annexure – III but not limited to parameter identified.

4.1.12 If a particular user point is showing out of specification result, check the particular user point for any leakage, rusting, breakage etc.

4.1.13 Check the sanitization data of water system.

4.1.14 If the results cannot be attributed to the analytical error, sampling error, contamination or cross contamination in the container sampled, the microorganism detected must be identified / differentiated by taking sample from all other points.

4.1.15 If cause identified / none identified, Head QA / Head Engineering / Plant Head collectively decide to perform following activity to be carried out but not limited to these.

Adequate sanitization of the system shall ensure to eliminate the source of contamination with rigorous check for the same.

Cleaning and sanitization of storage tank in case of potable water.

Cleaning and sanitization of storage tank in case of Purified water & sanitization of distribution loops.

4.1.16 After the completion of above activities water to be sampled from suspected end user point, return line and storage tank and analyze the same.

4.1.17 Hold (retain) all the batches manufactured from the date of sampling till the result of re-test has been observed.

4.1.18 Test the MLT for number of batches manufactured by using the suspected water.

If the result comes within specified limit for affected batches, raise the CAPA and batches can be released.

4.1.19 If the result found out of specified limit for affected batches, raise the CAPA batches shall be rejected.

Note: If count exceed “alert limit” inform to concern department through QA as per “alert action

limit intimation form" of SOP and only additional sanitization and cleaning of the water system to be carried out and no need to follow complete investigation procedure." If count observed more than "action limit" than raise the deviation and investigation shall be carried out to find out route cause, documented and corrective and preventive action shall be taken.

4.2 Failure investigation of Total aerobic microbial count and test for specified microorganisms testing:

4.2.1 The failure of MLT test results is indicated by the development of turbidity in the liquid media and growth on solid agar surface used in MLT testing.

4.2.2 If with in specified limit microbial growth is found, the product to be examined complies with the test for MLT.

4.2.3 If out of specified limit microbial growth is found, the product to be examined does not complies with the test for MLT.

4.2.4 Failure investigation carried out as described in the flow chart diagram as per annexure – II.

4.2.5 Laboratory Investigation shall be carried out as per annexure – IV but not to the parameter identified.

4.2.6 In case of assignable cause identified during the laboratory Investigation and if reanalysis required, in this case Material/product Resample/ Re analysis approval required from the Head QA after the detail justification from Head QC.

4.2.7 If Retesting approved from Head QA; Head Microbiologist allotted the sample to the analyst -1 and Repeat the Microbial Limit test of the batch with same number of samples. Hold (retain) the entire batch manufactured from the date till the result of re test has been observed.

4.2.8 If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for MLT. Complete the investigation of the invalidation of the discrete OOS result; raise CAPA before taking a decision to release the product/item.

4.2.9 If the growth is observed in repeat analysis whether identical/not identical to personnel or environmental monitoring data; raise the CAPA and batch will be rejected with no further sampling/testing required.

4.2.10 In the above case; OOS shall be hand over to QA for cross functional investigation for identify

route cause as mentioned in step No. 4.4.13.

4.2.11 If no assignable cause identified during laboratory investigation; proceed for cross functional investigation for identify route cause.

4.2.12 QA will investigate cross functional investigation and identified route cause as per annexure – IV. But not to be limited to the parameter identified.

4.2.13 Investigation shall be extended to previous and subsequent batches to find out the root cause and to prevent reoccurrence.

4.2.14 If route cause identified during cross functional investigation related to process, manpower, equipment, environment, material then raise CAPA complete the investigation of the confirmation of the OOS results/ product failure/affected batches and reject the product/ material.

4.2.15 If no assignable cause identified during laboratory as well as cross functional investigation Head QC or designee has to evaluate and requirement of re sampling with consulting Head QA.

4.2.16 Re sampling Authorization

4.2.17 For a new sample from the same batch lot of product or material may be considered in following cases after the approval of Head QA.

If case failure during initial investigation indicate a possible sampling error,

If insufficient quantity of the original sample remains to perform all further testing

When the original sample was not truly representative of the batch or there was a documented/traceable lab error in its preparation.

If the investigation determines that the initial sampling method was in error

Evidence indicates that the sample is compromised or invalid.

4.2.18 Re-sampling to be performed by the same methods that were used for the initial sample. If, the investigation determines that the initial sampling method was in error, a new accurate sampling method shall be developed, qualified and documented (relevant SOP for sampling shall be modified).

4.2.19 Based on above design continue the investigational analysis as below

4.2.20 Head QC or designee shall instruct Analyst 1 and 2 to retest the new portion with the resampling material (If sampling allowed)or from original sample (If Re sampling not required/ not

allowed)

4.2.21 If any of the test results does not comply with the specification, then raise CAPA complete the investigation of the confirmation of the OOS results/ product failure, and reject the product/ material.

4.2.22 If result of Analyst 1 & 2 complies with the MLT specification then raise CAPA complete the investigation of the invalidation of the discrete OOS result; before taking a decision to release the product/item.

4.3 Failure investigation of Environmental/Personnel Monitoring

4.3.1 The failure results are indicated by the development of growth on solid agar surface.

4.3.2 Environmental/personnel monitoring Failure investigation carried out as described in the flow chart diagram as per Annexure – II.

4.4 If result goes exceeds the Alert limit:

4.4.1 If observation is found out of alert limit immediately inform to Head Quality control, Quality Assurance and to the concerned department through alert and action limit intimation form of SOP for immediate additional cleaning and sanitization and for check and rectify any visual abnormalities in the concerned area / location / system.

4.5 If result goes out of Action limit:

4.5.1 Immediately inform to Head Quality control, Head production, Head QA through alert and action limit intimation form of SOP and quarantine the batches manufactured on the particular day. Inform the concerned department for immediate cleaning and sanitization of the concerned area / location observed.

4.5.2 Inform QA to quarantine the batches manufactured inline with the failure period of environmental/personnel monitoring. QA should initiate the full scale investigation to find out the exact cause of failure

4.5.3 Identify the colony characteristics of the bacteria / fungi observed and carry out identification as per SOP: Procedure for identification of microbial cultures/microbial isolates. If the test is showing the presence of pathogens, confirm it with confirmatory test.

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4.5.4 Laboratory Investigation shall be carried out as per annexure– V but not limited to parameter identified.

4.5.5 Investigation shall be extended to previous and subsequent A R Number to find out the root cause and to prevent reoccurrence.

4.5.6 If assignable cause identified during laboratory investigation, and if it is corrected through calculation then correct the same and report the results.

4.5.7 If assignable cause identified during laboratory investigation and if analysis is required to arrive at conclusion then QC Head, QA Head collectively decided to extent of the sampling and evaluates the impact / risk.

4.5.8 If no assignable cause identified during laboratory investigation, for further investigation hand over to QA.

4.5.9 QA will investigate cross functional investigation for identification route cause as per annexure – V but not limited to parameter identified.

4.5.10 In case of environmental failure; if a particular location is showing out of specification result, check the particular location area/system for any abnormalities.

4.5.11 If the results cannot be attributed to the analytical error, sampling error, contamination or cross contamination in the container sampled than the microorganism detected must be identified / differentiated by taking sample from all other points.

4.5.12 If cause identified / none identified, Head QA / Head Engineering / Plant Head collectively decide to perform following activity to be carried out but not limited to these.

Entry and exit record of concerned area (if applicable)

Cleaning and sanitization of the concern area / location.

Checking the HVAC system for any leakage/abnormality.

4.5.13 In case of Environmental monitoring failure; Hold (retain) all the batches manufactured from the date of sampling till the result of re-test has been observed.

4.5.14 In case of personnel monitoring failure; Hold (retain) all the batch/es associated with the concern personnel and risk assessment to be raise.

4.5.15 In case of environmental failure; retesting shall be carried out for three consecutive days

and based on the data, the area shall be released and handed over to production department for routine production activity.

4.5.16 Test for microbiological parameter (MLT) for number of batches manufactured in the concern area in which failure result in environmental monitoring test.

4.5.17 If the result comes within specified limit for affected batches, raise the CAPA batches can be released.

4.5.18 If the result found out of specified limit for affected batches, raise the CAPA batches shall be rejected.

Note: If count exceed "alert limit" inform to concern department through QA as per "alert action limit intimation form" of SOP and additional sanitization and cleaning of the area to be carried out and no need to follow complete investigation procedure". If count observed more than "action limit" than raise the deviation and investigation shall be carried out to find out the route cause, documented and corrective and preventive action shall be taken. Environmental Monitoring investigation includes active air sampling (air sampling), passive air sampling (settle plate method) and surface monitoring.

4.6 Failure investigation of Compressed air / Nitrogen gas:

4.6.1 The failure results are indicated by the development of growth on solid agar (in case of microbial enumeration) surface.

4.6.2 Compressed air / Nitrogen gas Failure investigation carried out as described in the flow chart diagram as per Annexure – II.

4.6.3 If the counts exceeds, analyst shall inform to senior immediately. Senior Immediately inform to Head Quality control and Head QA and to the concerned department.

4.6.4 Identify the colony characteristics of the bacteria / fungi observed and carry out identification as per SOP.: Procedure for identification of microbial cultures/microbial isolates If the test is showing the presence of pathogens, confirm it with confirmatory test.

4.6.5 Inform QA to quarantine the batches manufactured during the Compressed air / Nitrogen gas failure period. QA should initiate the full scale investigation to find out the exact cause of failure

4.6.6 Laboratory Investigation shall be carried out as per annexure–VI but not limited to the parameter identified.

4.6.7 Investigation shall be extended to previous and subsequent A. R. Number to find out the root cause and to prevent reoccurrence.

4.6.8 If assignable cause identified during laboratory investigation, and if it is corrected through calculation then correct the same and report the results.

4.6.9 If assignable cause identified during laboratory investigation and if analysis is required to arrive at conclusion, QC Head, QA Head collectively decided to extent of the sampling and evaluates the impact / risk.

4.6.10 If no assignable cause identified during laboratory investigation, for further investigation hand over to QA.

4.6.11 QA will investigate cross functional investigation for identification of route cause as per Annexure –VI but not limited to the parameter identified.

4.6.12 If a particular user point is showing out of specification result, check the particular user point for any leakage, rusting, breakage etc.

4.6.13 If the results cannot be attributed to the analytical error, sampling error, contamination or cross contamination in the container sampled, than microorganism detected must be identified / differentiated by taking sample from all other points.

4.6.14 If cause identified / not identified, Head QA / Head Engineering / Plant Head collectively

decide to perform following activity to be carried out but not limited to these.

Check for any leakage or breakage of compressed air / nitrogen gas.

Check the filer integrity (if applicable) for filter attached with particular user/generation end point.

4.6.15 After the completion of above activities compress air / nitrogen gas to be sampled from suspected end user point.

4.6.16 Hold (retain) all the batches manufactured from the date of sampling till the result of re-test has been observed.

4.6.17 Test the number of batches manufactured for MLT by using the suspected compressed air and nitrogen gas.

4.6.18 If the result comes within specified limit, raise the CAPA batches can be released.

4.6.19 If the result found out of specified limit, raise the CAPA batch shall be rejected

4.7 For Microbial Assay investigation to be carried out as per reference SOP.

4.8 Failure investigation of Growth Promotion test:

4.8.1 If growth promotion test of microbiological media fails than raise the deviation and Growth Promotion Failure investigation carried out as described in the flow chart diagram as per Annexure – II.

4.8.2 Laboratory Investigation shall be carried out in line with the failure observed in GPT as per Annexure – VII but not limited to parameter identified.

4.8.3 A laboratory investigation shall be carried out immediately which shall include

Errors in calculation

Error in analysis.

Error in sampling, handling and storage of sample

4.8.4 Check the number of samples analyzed by the analyst along with the sample of suspected OOS.

4.8.5 If laboratory investigation reveals that out of specification is due to calculation error then correct the calculation and stop further investigation.

4.8.6 In case of assignable cause identified during the laboratory Investigation and if reanalysis required, in this case Microbiological Media Resample/ Re analysis approval required from the Head QA after the detail justification from Head QC.

4.8.7 If Retesting approved from Head QA; Head Microbiologist allotted the microbiological culture media sample to the analyst -1 and Repeat the test of the batch with same quantity of media. Hold (retain) the entire batch manufactured from the date till the result of re test has been observed.

4.8.8 If growth is observed in the repeat test, the microbiological culture media examined complies with the test for growth promotion. Complete the investigation of the invalidation of the discrete OOS result; raise CAPA before taking a decision to release the media.

4.8.9 If the growth is not observed in repeat analysis; raise the CAPA and media batch will be rejected with no further sampling/testing required.

4.8.10 In the above case; OOS shall be hand over to QA for cross functional investigation for identify route cause as mentioned in step No.4.9.10.

4.8.11 If no assignable cause identified during laboratory investigation; proceed for cross functional investigation to identify route cause.

4.8.12 QA will investigate cross functional investigation and identified route cause as per Annexure – VII but not to the parameter identified.

4.8.13 Investigation shall be extended to previous and subsequent batches to find out the root cause and to prevent reoccurrence.

4.8.14 If route cause identified during cross functional investigation related to process, manpower, equipment, environment, material, measurement: then raise CAPA complete the investigation for the confirmation of the OOS results/ product failure/affected batches and reject the product/ material.

4.8.15 If no assignable cause identified during laboratory as well as cross functional investigation Head QC or designee has to evaluate and requirement of re sampling with consulting

Head QA.

4.9 Re sampling Authorization:

4.9.1 For a new sample for growth promotion test from the same batch lot of product or material may be considered in following cases after the approval of Head QA.

If case failure during initial investigation indicate a possible sampling error,

If insufficient quantity of the original sample remains to perform all further testing

When the original sample was not truly representative of the batch or there was a documented/traceable lab error in its preparation.

Evidence indicates that the sample is compromised or invalid.

4.9.2 Re-sampling to be performed by the same methods that were used for the initial sample. If, the investigation determines that the initial sampling method was in error, a new accurate sampling method shall be developed, qualified and documented. (Relevant SOP for sampling shall be modified).

4.9.3 Based on above design continue the investigational analysis as below

4.9.4 Head QC or designee shall instruct Analyst 1 and 2 to retest the new portion with the resampling material (If sampling allowed)or from original sample (If Re sampling not required/ not allowed)

4.9.5 If any of the test results does not comply with the specification, then raise CAPA complete the investigation of the confirmation of the OOS results/ product failure, and reject the product/ material and return media to the supplier.

4.9.6 If result of Analyst 1 & 2 complies with the GPT specification then raise CAPA complete the investigation of the invalidation of the discrete OOS result before taking a decision to release the product/item.

5.0 ANNEXURE (S):

Annexure–I: Out of Specification Investigation Form Issuance Register.

Annexure-II: Flow chart diagram of investigation of out of specification.

Annexure-III: Investigation of out of specification of Water test results.

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Annexure- IV: Total aerobic microbial count and Test for specified Microorganisms failure investigation.

Annexure-V: Investigation of out of specification of Environmental/Personnel Monitoring test results.

Annexure- VI: Investigation of out of specification of Compressed air & Nitrogen gas test results.

Annexure- VII: Investigation of out of specification of Growth Promotion test result.

6.0 REFERENCE (S):

SOP: Procedure for identification of microbial cultures/microbial Isolates.

SOP: Investigation of out of specification of test results in microbiology.

SOP: Handling of Non Conformance

SOP: Sampling and Analysis of Water

SOP: Preparation, Approval, Distribution control, revision and Destruction of Standard Operating Procedure (SOP).

7.0 ABBREVIATION (S) / DEFINITION (S):

HEPA: High efficiency particulate air filter.

MLT : Microbial Limit Test

LAF : Laminar Air flow.

GPT : Growth promotion test

IP : Indian Pharmacopoeia

EP : European Pharmacopoeia

BP : British Pharmacopoeia

USP : United State Pharmacopoeia

SOP : Standard Operating Procedure

QCM : Quality Control Microbiology

i.e. : That is

v/v : Volume by Volume

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