Title: Handling of Out of Limit Results in Environmental Monitoring and Water Analysis			
SOP No.:		**Revision No.:**	00
Effective Date:		**Supersedes No.**	Nil
Review Date:		**Page No.**	1 of 17

1.0 PURPOSE:

To lay down procedure for handling of out of limits (OOL) results of environmental monitoring and water system monitoring results.

2.0 SCOPE:

Applicable to handling and conducting investigations when out of limit results are obtained in environmental monitoring and water system monitoring of production and microbiology laboratory facilities.

3.0 RESPONSIBILITY

3.1 Quality Control Microbiology

Microbiology Officer is responsible to notify Microbiology Head or his designee when and alert or action limit is obtained.

Head - Microbiology or his designee is responsible to notify the QA and concerned departments and initiate investigation in the laboratory and concerned department.

To implement any corrective action and preventive action

3.2 Quality Assurance Department

To participate in investigations

To review and approve investigation reports

To review action taken reports

3.3 Production / Engineering Department

To participate in investigations

To implement necessary corrective and preventive actions

4.0 PROCEDURE

4.1 Notification and allotment of number for Out Of Limit Result

4.1.1 On obtaining an out of limit result, the microbiologist shall notify the Microbiology In - Charge or his designee and they shall notify the QA and concerned department.

4.1.2 The details of Out of Limit results shall be entered in Log Book (Annexure – VI) and a number shall be allotted as M-OOL/YY/ZZZ, where
M-OOL : Microbiological Monitoring – Out Of Limit Result
YY : Represents last two digits of current year
ZZZ : Represents serial number starting with 001
Example : First OOL reported in year 2024: M-OOL/24/001

4.2 Handling and Investigation of Out of Limits Results in Microbiological Monitoring of Clean rooms

Note: This section is applicable for handling and investigation of out of limit results in Passive Air Sampling (Settle Plate), Active Air Sampling and Surface Sampling.

4.2.1 Observe the plates under incubation (sampled after the date of sampling showing out of limit results) of the sample location / room showing out of limits and check of for any out of limit results. Inform the observations to Head Microbiology or his designee.

4.2.2 Verify the negative control plate incubated along with test samples for any contamination. Inform the observation to Head Microbiology or his designee.

4.2.3 Process the out of limit results for identification as follows:

- In case of out of alert limit results, perform gram staining of morphologically similar colonies and identification of representative isolates based on Gram Staining Results as per SOP.
- In case of out of action limit results in Grade C and Grade D areas, perform gram staining of morphologically similar colonies and identification of representative isolates based on Gram Staining Results as per SOP.
- In case of out of action limit results in Grade A and Grade B areas perform identification of all isolates as per SOP and also by DNA sequencing.

4.2.4 <u>Investigation of Out of Alert Limit Results</u>

4.2.4.1 Review the data for the sample location/room in question for any previous instances of out of limit results in last three months.

4.2.4.2 If review of data or plates under incubation shows occurrences of out of alert limit results for more than three consecutive days or occurs frequently then the investigation should be elevated to out of action limits.

4.2.4.3 Laboratory Investigation

- Interview the microbiologist who performed sampling and verify whether the sampling was performed as per SOP and if any deviations observed during the sampling/testing/transportation and disinfection of plates.
- Verify the instruments used (air sampler) were operated properly and accessories used (air sampler head or swab template) were sterilized.
- Verify all the media used were within their shelf life and review their preparation records for results of pre-incubation and sterility checks.
- Interview the media personnel for any deviation observed during preparation/pouring of plates.
- Review the results of all EMP parameter of the particular days.(Active, Passive ,surface & personnel monitoring)
- If contamination in negative control plates is observed or laboratory investigation reveals fault in sampling, discrepancies in status of air sampler, air sampler head/swab templates or results of media pre-incubation and sterility check are not satisfactory, then the occurrence of out of limit results could be attributed to laboratory/sampling error.

4.2.4.4 Facility Investigation

- Review cleaning/disinfection logs, operational logs and other activities of subject area for any discrepancies.
- If the sample location is in critical area, then review the entry exit logs for number of persons present in the area, their duration of stay in the area and their personal hygiene and training / qualification status.
- Review of records of physical conditions like pressure differentials, temperature and relative humidity of the subject area on the day, days before and after occurrence.
- Contact Engineering department for any discrepancies in the functioning of HVAC and other systems or any maintenance activities undertaken or due for maintenance
.
- If any discrepancies observed during review, determine if it has any impact on the observed results.
- Record all the observations in the investigation report.

4.2.4.5 Based on the information gathered, determine if follow up monitoring is required or not as per Investigation report (Annexure I) and proceed accordingly.

4.2.4.6 If the results of the follow-up monitoring performed are not satisfactory elevate the investigation to out of action limit investigations.

4.2.4.7 Review the identification results and verify if it is normal micro flora of the area. If isolate is different or objectionable, initiate necessary corrective actions.

4.2.5 Investigation of Out of Action Limit Results

4.2.5.1 Based on the criticality of operations performed in the area showing out of limit results and observations of plates under incubation, decision shall be taken for use of the area for critical operations. If out of action limits are observed in Grade A and B of filling area then investigation to be trigged. Based on the risk, the production shall be stopped till completion of investigation and after obtaining satisfactory results of three consecutive days, given the clearance to start the production by Head QA with the consultation of Head Engineering and Head Production.

4.2.5.3 Quarantine the subject batches till investigation is complete, based upon the investigation & risk assessment if finding impact on product quality the concern batches shall be rejected and if finding no impact on product quality then concern batches shall be release.

4.2.5.2 Review the data for the sample location/room in question for any previous instances of out of limit results in last three months.

4.2.5.3 If the data indicates previous occurrences of out of limits, then review the previous investigation reports to determine any similarities.

4.2.5.4 Laboratory Investigation

- Interview the microbiologist who performed sampling and verify whether the sampling was performed as per SOP and if any deviations observed during the sampling/testing/transportation and disinfection of plates.
- Verify the instruments used (air sampler) were operated properly and accessories used (air sampler head or swab template) were sterilized.
- Verify all the media used were within their shelf life and review their preparation records for results of pre-incubation and sterility checks.
- Interview the media personnel for any deviation observed during preparation/pouring of plates.

- Review the results of all EMP parameter of the particular days.(Active, Passive ,surface & personnel monitoring)

- If contamination in negative control plates is observed or laboratory investigation reveals fault in sampling, discrepancies in status of air sampler, air sampler head/swab templates or results of media pre-incubation and sterility check are not satisfactory, then the occurrence of out of limit results could be attributed to laboratory/sampling error.

4.2.5.5 Facility Investigation

- Review cleaning / disinfection logs, operational and other activities of subject area for any discrepancies.

- If the sample location is in critical area, then review the entry exit logs for number of persons present in the area, their duration of stay in the area and their personal hygiene and training / qualification status.

- Interview the personnel's of particular day in which the OOL Observed for any deviation observed during gowning procedure and practices in area etc..

- Review the material movement procedure and any other deviation /Change in procedure

- Review of records of physical conditions like pressure differentials, temperature and relative humidity of the subject area on the day, days before and after occurrence.

- Review the preparation and sterilization records of materials used in the area for any deviations.

- If the action limit has occurred during batch activity, review the executed batch record for any discrepancies or other helpful information. Review the batches manufactured during occurrence of out of action limit results for microbiological parameters.

- Contact Engineering department for any discrepancies in the functioning of HVAC (Velocity, air change & HEPA filter integrity) and other systems or any maintenance activities undertaken or due for maintenance.

- Review the non-viable particulate count results of the particular area performed on the day, days before and after occurrence.

- Visit the subject area and verify the physical conditions, general cleanliness and any other

abnormalities, which could have contributed for the occurrence of out of action limit results.

- If any discrepancies observed during, determine if it has any impact on the observed results.

4.2.5.6 Based on the information gathered, determine if follow up monitoring is required or not as per Investigation report (Annexure I) and proceed accordingly.

4.2.5.7 If no assignable cause is identified or the follow up monitoring results are not satisfactory, in addition to above actions, appropriate additional measures can be initiated as follows:

- Increasing of cleaning/disinfection or change of disinfectants.
- Increasing in monitoring frequencies or increase of sample points in subject area for monitoring.
- Testing for non-viable particulate counts.
- Testing of HEPA filters for integrity and air velocity
- Any other appropriate activity

4.2.5.8 Review the identification results and verify if it is normal micro flora of the area. If isolate is different or objectionable, initiate necessary corrective actions.

4.3 Handling and Investigation of Out of Limits Results in Non Viable Monitoring

Note: This section is applicable for handling, out of limit results of Microbiological lab facility.

4.3.1 On obtaining any out of limit results during non-viable monitoring, immediately do the following:

4.3.2 Check the instrument is operating properly and any disturbance or changes in room conditions is observed.

4.3.3 Check activities (specifically for particle or aerosol generating or disturbance to particle counter probe) performed around the sample location during the time of out of limit result and evaluate if it has any effect on the reported result.

4.3.4 Verify that the instrument used was within calibration and testing performed as per procedure. If appropriate perform the zero count of the particle counter.

4.3.5 Resample the location after the conditions are restored and verify the results. Record the noted observation the report. If the results of resample are conforming to limits, then no further action is required. If the results are still non-conforming proceed to 4.3.6

4.3.6 Review the trend for the sample location / room in question and results of other sample locations performed on the day.

4.3.7 Review department cleaning logs, room differential pressure records, number for personal in the room at the time of testing, number of equipments and their operation status and other activities for any discrepancies.

4.3.8 Contact Engineering / Maintenance department and review logs for any discrepancies in the functioning and maintenance of HVAC and other systems or any maintenance activities undertaken.

4.3.9 Evaluate the information gathered and determine if it has an impact on the results observed.

4.3.10 Based on the information gathered, evaluate the actions to be performed and perform re-sampling of the concerned location or room as per Investigation report (Annexure V) and proceed accordingly.

4.3.11 If the resample results conform to limits then no further action is required.

4.3.12 If the resample result does not conform to limits, then carry out further investigation for determining the root cause. Following activities can be performed to determine the root cause:

- Extensive cleaning of area
- Air Velocity verification of HEPA filters
- HEPA filter integrity testing
- Air flow studies

4.4 Handling and Investigation of Out of Limits Results in Personnel Monitoring

4.4.1 Observe the plates under incubation (sampled after the date of sampling showing out of limit results) of the sample same person(s) showing out of limits and check of for any out of limit results. Inform the observations to Head Microbiology or his designee.

4.4.2 Verify the negative control plate incubated along with test samples for any contamination. Inform the observation to Head Microbiology or his designee.

4.4.3 Observe the other plates of environmental monitoring performed on the day of out of limit occurrence and compare the colonies with plates showing out of limit results.

4.4.4 Process the out of limit results for identification along with morphologically similar colonies from environmental monitoring plates if any as follows:

4.4.4.1 Perform gram staining of morphologically similar colonies and identification of representative isolates based on Gram Staining Results as per SOP.

4.4.4.2 In case of out of limit results in Grade A and Grade B areas perform identification of all isolates as

per SOP and also by DNA sequencing.

4.4.5 Laboratory Investigation

4.4.5.1 Interview the microbiologist who performed sampling and verify whether the sampling was performed as per SOP and if any deviations observed during the sampling/testing.

4.4.5.2 Verify all the media used were within their shelf life and review their preparation records for results of pre-incubation and sterility checks.

4.4.5.3 If contamination in negative control plates is observed or laboratory investigation reveals fault in sampling, discrepancies in results of media pre-incubation and sterility check are not satisfactory, then the occurrence of out of limit results could be attributed to laboratory/sampling error.

4.4.6 Facility Investigation

4.4.6.1 Review the previous data of concerned person for any out of limit results.

4.4.6.2 Verify the personal hygiene and health status of concerned person. Review the medical and training records of the concerned person.

4.4.6.3 Review the environmental monitoring data of day and days before and after the day of occurrence for out of limit results.

4.4.6.4 Review cleaning / disinfection logs, entry exits logs and other activities of subject area for any discrepancies.

4.4.6.5 Review of records of physical conditions like pressure differentials, temperature and relative humidity of the subject area on the day, days before and after occurrence.

4.4.6.6 Review the garment preparation and sterilization records for any discrepancies.

4.4.7 Based on the information gathered, determine the actions to performed as per Investigation and Decision Flow Chart in (Annexure I) and proceed accordingly.

4.4.8 Review the activities performed the concerned individual and if he has performed critical aseptic operations, critically review the microbiological results of the concerned batch.

4.5 Handling and Investigation of Out of Limits Results in Microbiological Analysis of

Water

4.5.1 Observe the plates of same type of water sampled and analyzed on the same day and those under incubation (sampled after the date of sampling showing out of limit results) of the sample location / sample type showing out of limits and check of for any out of limit results. Inform the observations to Head Microbiology or his designee.

4.5.2 Verify the negative control plate incubated along with test samples for any contamination. Inform the observation to Head Microbiology or his designee.

4.5.3 Process the out of limit results for identification as follows:

- In case of out of alert limit results, perform gram staining of morphologically similar colonies and identification of representative isolates based on Gram Staining Results as per SOP.
- In case of out of action limit results, perform gram staining and identification of all colonies for WFI and Pure Steam Condensate and morphologically similar colonies isolates based on Gram Staining Results for Purified Water and other water samples as per SOP.

4.5.4 Investigation of Out of Alert Limit Results

4.5.4.1 Review the data for the sample location / system in question for any previous instances of out of limit results in last three months.

4.5.4.2 If the data indicates previous occurrences of out of limits, then review the previous investigation reports to determine any similarities.

4.5.4.3 Laboratory Investigation

- Interview the microbiologist who performed sampling and verify whether the sampling was performed as per SOP and if any deviations observed during the sampling/testing.
- Verify the materials used for sampling and testing was properly sterilized and handled.
- Verify all the media used were within their shelf life and review their preparation records for results of pre-incubation and sterility checks.
- If contamination in negative control plates is observed or laboratory investigation reveals fault in sampling and testing results of media pre-incubation and sterility check

are not satisfactory, then the occurrence of out of limit results could be attributed to laboratory/sampling error.

4.5.4.4 Water System / Facility Investigation

- Contact Engineering and Production Department for any discrepancies in the functioning of water systems or any maintenance undertaken. Verify the operation and sanitization log books for concerned system / area.

- If the out of limit result is observed in only one sample location and rest of the system is conforming to specifications, then verify the sample location for any discrepancies in the sample/user point and the location.

4.5.4.5 Based on the information gathered, determine the actions to be initiated as per Investigation report in Annexure IV and proceed accordingly.

4.5.4.6 Review the identification results and verify if it is normal micro flora of the water system or of human commensal or from environment. If isolate is different or objectionable, initiate necessary corrective actions.

4.5.4.7 If the results of follow up sampling are satisfactory after carrying out corrective actions (if any) conclude the investigation.

4.5.4.8 If the results of follow up sampling are not satisfactory carry out further investigation take necessary actions accordingly.

4.5.5 Investigation of Out of Action Limit Results

Note: This is also applicable for any non-conformance to test for specified organisms.

4.5.5.1 Review the data for the sample location / system in question for any previous instances of out of limit results in last three months.

4.5.5.2 If the data indicates previous occurrences of out of limits, then review the previous investigation reports to determine any similarities.

4.5.5.3 If the subject sampling location is a daily monitoring sample location and results of subsequent days are also showing out of limit results or the sample location is on sampling rotation, them immediately schedule for three consecutive day sampling.

4.5.5.4 Laboratory Investigation

- Interview the microbiologist who performed sampling and verify whether the sampling was performed as per SOP and if any deviations observed during the sampling/testing.
- Verify the materials used for sampling and testing was properly sterilized and handled.
- Verify all the media used were within their shelf life and review their preparation records for results of pre-incubation and sterility checks.
- If contamination in negative control plates is observed or laboratory investigation reveals fault in sampling and testing results of media pre-incubation and sterility check are not satisfactory, then the occurrence of out of limit results could be attributed to laboratory/sampling error.

4.5.5.5 Water System / Facility Investigation

- Contact Engineering and Production Department for any discrepancies in the functioning of water systems or any maintenance undertaken. Verify the operation and sanitization log books for concerned system / area.
- If the out of limit result is observed in only one sample location and rest of the system is conforming to specifications, then verify the sample location for any discrepancies in the sample/user point and the location.
- If the water from the specific location was used for batch manufacturing, then verify the results in-process and finished product samples of concerned batch(es).
  - Bio-burden results of bulk sample before filtration
  - Microbiological tests results of Oral Solid Dosages

4.5.5.6 Evaluate the information gathered and determine if it has any impact on the observed results.

4.5.5.7 Based on the information gathered, determine the actions to be initiated as per Investigation report (Annexure IV) and proceed accordingly.

Review the identification results and verify if it is normal micro flora. If isolate is different, then include it micro flora stock for use in different tests.

If isolate identified is of objectionable, then investigate the possible source of

contamination and take necessary corrective and preventive actions.

If the results of follow up sampling are satisfactory after carrying out corrective actions (if any) conclude the investigation.

If the results of follow up sampling are not satisfactory carry out further investigation take necessary actions accordingly. Following activities can be performed to determine the root cause:

- Sampling and analysis at different stages of generation and distribution system to identify the contamination.
- Sanitization of generation, storage, distribution and heat exchangers as applicable.
- Verification of air vent filters.
- Verification of gaskets, valves and other components.

4.5.5.8 The system can be released for use after obtaining satisfactory results for consecutive three days.

4.6 Investigation of Out of Limits Results in Chemical Analysis, BET of Water

4.6..1 On obtaining out of limit results in any chemical analysis (except for TOC) and BET, inform to Head Microbiology. Do not discard the original left over sample (if any).

4.6.2. Verify the status (cleaning or depyrogenation) of the glassware used for sampling and testing.

4.6.3 Verify the status of the chemicals, reagents and instruments used in the analysis.

4.6.4 Verify the test is performed properly as per procedure for any analyst error during testing.

4.6.5 If any discrepancy is observed in glassware used for sampling and testing, then take necessary corrective actions and arrange for re-sampling from particular sample point from glassware conforming to requirements.

4.6.6 If the any discrepancy is observed in chemicals, reagents or instruments, then take necessary corrective actions and retest using original sample if available or with fresh sample.

4.6.7 If the analyst error is observed, then second analyst shall perform the test.

4.6.8 If the results of the resample conform to specifications, then record the results and water may be released.

4.6.9 If the no assignable cause is determined above or the test results show non-conformance on re-sampling and testing, then inform the concerned department and QA.

4.6.10 Perform the investigation to determine the root cause and take necessary corrective actions. Following activities can be performed to determine the root cause:

- Testing of input water and at different stages in the treatment and generation system.
- Cleaning and sanitization of generation, storage and distribution system.

4.6.11 The system can be released for use after obtaining satisfactory results for consecutive three days.

4.6.12 For investigation of chemical analysis follow Annexure-II and for BET follow Annexure –III.

4.7 Investigation of Out of Limit Results in TOC

4.7.1 **Investigation out of Alert Limit Results**

4.7.1.1 If TOC results exceed the above Alert level then the following actions shall be initiated.

4.7.1.2 Any samples exceeding the alert limits shall be immediately informed to the Head Microbiology & QA and do not discard the original left over sample (if any)

4.7.1.3 Review the data for the sample location /system in question for any previous instances of out of limit results in last three months.

4.7.1.4 If the last data indicates previous occurrences of out of limits, then review the previous Investigation reports to determine any similarities.

4.7.1.5 Laboratory Investigation

- Interview the microbiologist who performed sampling and verify whether the sampling was performed as per sop and if any deviation observed during the sampling/testing.

- Verify the glassware used for sampling was properly cleaned.

- Verify that sample was intact during transportation.
- Verify that the instrument used was within calibration and testing performed as per procedure.

4.7.1.3 If laboratory investigation reveals fault in sampling/glassware used/and transportation Then the occurrence of out of limit results could be attributed to laboratory /sampling error.

4.7.1.4 An immediate repeat test of the original sample together with an additional sample from the same location shall be performed.

4.7.1.5 If the TOC resample show results over the alert limit then additional user points shall be immediately sampled and tested.

4.7.1.6

4.7.1.7 If results suggest that only one point is affected and it is an isolated incident then the result will be recorded and used for trending analysis & investigate for corrective and preventive action.

4.7.2 If the retest results from the additional user points are found out of Alert limit, the results shall be informed to Head QA, Production and Engineering department to carry out the detailed investigation and take immediate corrective and preventive actions as per Annexure-II.

4.7.2.1 **Investigation out of Action Limit Results.**

4.7.2.2 If TOC results exceed the above Action level then the following actions shall be initiated.

4.7.2.3 Any Samples exceeding the action limits shall be immediately informed to the Head Microbiology & do not discard the original left over sample (if any)

4.7.2.4 Review the data for the sample location /system in question for any previous instances of out of limit results in last three months.

If the last data indicates previous occurrences of out of limits, then review the previous Investigation reports to determine any similarities.

<u>Laboratory Investigation</u>

4.7.2.5 Interview the microbiologist who performed sampling and verify whether the sampling was performed as per sop and if any deviation observed during the sampling/testing.

- Verify the glassware used for sampling was properly cleaned.
- Verify that sample was intact during transportation.
- Verify that the instrument used was within calibration and testing performed as per procedure.
- An immediate repeat test of the original sample together with an additional sample from the same location shall be performed.

4.7.2.6 If the TOC resample show results over the action limit then additional user points shall be immediately sampled and tested.

4.7.2.7

4.7.2.8 If results suggest that only one point is affected and it is an isolated incident Investigation shall be initiated to identify the root cause.

4.7.2.9 If the retest results from the additional user points are found out of Action limit, the results shall be informed to Head QA, Production and Engineering department to carry out the detailed investigation and take immediate corrective and preventive actions as per annexure-II

4.7.2.10 Until and unless the investigation is complete, and immediate corrective actions is completed no further batches will be manufactured.

4.7.2.11 The system can be released for use after investigation & obtaining the satisfactory results.

4.8 A trend of OOL shell be prepared and review for repetitive nature & Effectiveness of CAPA on half yearly basis.

5.0 ABBREVIATIONS & DEFINITIONS

SOP- Standard Operating Procedure
QCM- Quality Control Micro
Rev. - Revision
OOL- Out of Limit
OOS – Out of Specification

TOC- Total Organic Carbon

BET - Bacterial Endotoxin Test

Ltd. - Limited

No. - Number

CAPA- Corrective and preventive action

Action Level: Established criteria, requiring immediate follow-up and corrective action if exceeded

Alert Level: established criteria giving early warning of potential drift from normal conditions which are not necessarily grounds for definitive corrective action but which require follow-up investigation.

OOL: Any results obtained above the predefined alert and action level.

6.0 REFERENCE DOCUMENTS

SOP: Microbial Identification by VITEK2 Compact Identification

7.0 ANNEXURE/ATTACHMENTS

Annexure I: Out of limits investigation report - Environmental monitoring.

Annexure II: Out of limits investigation report - Chemical analysis of water.

Annexure III: Out of limits investigation report - Bacterial endotoxin test of water

Annexure IV: Out of limits investigation report - Microbiological testing of water

Annexure V: Out of limits investigation report – Non-viable Monitoring

Annexure VI: Microbiology testing out of limit log

8.0 REVISION LOG

Revision Number	Effective Date	Reference Change Control No	Reason for Revision