PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Handling of Out of Specification results for Microbiological Analysis

SOP No.:		**Department:**	Microbiology	
		Effective Date:		
Revision No.:	00	**Revision Date:**		
Supersede Revision No.:	Nil	**Page No.:**	1 of 20	

1. Purpose: The purpose of this SOP is to define the procedure for the investigation of out of specification results for microbiological analysis in Quality Control departments.

2. Scope:

2.1. This guideline is applicable to OOS results generated for microbiological analysis and pathogens of raw material, finish product, stability samples.

2.2. In case of questionable / failure results obtained for environmental monitoring and water testing results shall be handled as per below guideline.

2.3. Microbial environmental monitoring results shall be handled through microbial environmental monitoring as per SOP.

2.4. Water testing results shall be handled through quality monitoring of water as per SOP.

3. References, Attachments & Annexures:

3.1. **References:**

3.1.1. In House

3.1.2. SOP Microbiological Environmental Monitoring

3.1.3. SOP Quality Monitoring of Water

3.1.4. SOP Event Reporting and Investigation

3.1.5. SOP Product recall

3.2. **Attachments:**

3.2.1. Attachment-1: Out of specification investigation form (Phase-I)

3.2.2. Attachment-2: Out of specification investigation form (Phase-II).

3.2.3. Attachment-3: Instruction note for trial/study

3.2.4. Attachment-4: Flow chart for OOS investigation.

3.2.5. Attachment-5: Flow chart for handling of human error

3.3. **Annexures:** None

4. Responsibilities:

4.1. **Microbiologist:**

4.1.1. To perform the activity as per SOP.

4.1.2. To maintain all the records as per SOP.

4.2. **QC Head or designee:**
- 4.2.1. To check the SOP.
- 4.2.2. To give training to all concerned persons before implementation of SOP.

4.3. **Quality Assurance:**
- 4.3.1. To check the SOP.
- 4.3.2. To ensure the implementation of system as per SOP.

4.4. **Regulatory Affairs, Quality Head , Plant Head:**
- 4.4.1. To approve the SOP.

5. Distributions:

5.1. Quality Control (Microbiology)

5.2. Quality Assurance

6. Definitions of terms & Abbreviations:

6.1. **Definitions of terms:**

6.1.1. **Evaluation samples :** Samples, which are of deviation batch, recovery batch, market complaint, any sample which is for study and trial purpose.

6.1.2. **OOS result:** A result, which is not confirmed but out of limit at the first analysis considerd as OOS and need to be investigated.

6.1.3. **Assignable cause:** A cause that has been identified as the reason to invalidate a test result.The assignable cause is a conclusion derived from direct or indirect evidence found during the investigation process, from the interpretation of analytical data,or a combination of both.

6.1.4. **OOS:** An unacceptable result that is out come of analysis. The result which does not meet the pre-established specification of the product shall be termed as OOS.

6.1.5. **Reanalysis:** Repeat the analysis by repeating one or more steps of the sample preparation from the original preparation or sample. A reanalysis may include the preparation of fresh standards and/ or other test reagents as appropriate.

6.1.6. **Re-sampling :** A re-sample is a repeating of the entire sampling procedure for defined product or item.

6.1.7. **Root cause analysis :** A structure documented investigation that aims to identify the true cause of failure or deviation and corrective and preventive actions necessary to eliminate it and prevent re-occurrence.

6.1.8. **CAPA (Corrective actions and preventive actions):** The action taken to correct the existing problem on non conformity and the action taken to prevent nonoccurence of a quality problem.

6.1.9. **Analyst-1:** First analyst by whom the OOS result is generated.

6.1.10. **Analyst-2:** Any analyst other than analyst-1 having more analytical experience.

6.2. **Abbreviations:**

6.2.1. **SOP** : Standard Operating Procedure
6.2.2. **No.** : Number
6.2.3. **QA** : Quality Assurance
6.2.4. **QC** : Quality Control
6.2.5. **NA** : Not Applicable
6.2.6. **OOS** : Out of Specification
6.2.7. **MLT** : Microbial Limit Test
6.2.8. **CAPA** : Corrective Action and Preventive Action

7. Procedure:

Note: The purpose of investigation is to determine the cause of OOS. The investigation shall be thorough, timely, unbiased,well documented and scientifically justifiable.

7.1. **General:**

7.1.1. In case of OOS result, analyst shall not destroy the original sample and kept under control environments till final disposition of analysis, where it is possible.

7.1.2. **OOS Investigation Phase-I:**

7.1.2.1. In case of observation of OOS results, analysts (Analyst-1) shall report to section head or designee.

7.1.2.2. Section head or designee shall inform to designated QA of QC person and Head QC, further designated QA of QC person shall take following actions.

7.1.2.3. Issue the "Out of specification investigation form (Phase-I)"to the analyst and make entry in the "Out of specification investigation form issuance logbook" as per Attachment -1 and separate register shall be maintained for OOS form issued for microbiology samples.

7.1.2.4. OOS Form No. for microbiology samples shall be given as M/001/2024, Where M stand for micro , 001 stands for serial number and 2024 stands for current year (e.g. First OOS Form No. in 2024 shall be given as M/001/2024.) "

7.1.2.5. Section head or designee shall carry out the investigation as per the "Details of the investigation phase-I" checklist but not limited to check points provided in the OOS investigation form.

7.1.2.6. Also section head or designee shall discuss with analyst and mention findings to find out the cause of OOS result.

7.1.2.7. Based on the investigation, section head or designee shall mention his remark and conclusion in OOS investigation form, which shall be reviewed by "QA of

QC" and shall be signed off.

7.1.2.8. Based on investigation, if any assignable cause found then proceed as per step no.7.1.3, phase -I.

If assignable cause is not found then proceed as per step no. 7.2 of OOS investigation phase-II.

7.1.3. **If assignable cause found :**

7.1.3.1. If assignable cause found during above investigation then invalidate the initial results and repeat test shall be carried out on same (or new sample) sample by analyst-1.

7.1.3.2. If sample passes, release the material / product.

7.1.3.3. If sample fails, then proceed as per step no. 7.1.4

7.1.4. Analyse the same laboratory sample by analyst-2, single time to confirm the error due to environment, media, practice and accessories used.

If sample passes, then follow the step no. 7.1.4.1

If sample fails, then re-sampling shall be carried out to rule out the sampling error and follow the step no. 7.2 of OOS investigation phase-II.

Note: If sample passes by analyst-2, then analytical practice of analyst-1 to be evaluated and shall be documented.

7.1.4.1. Head QC or designee shall instruct analyst-1 to analyse the same laboratory sample (or new) in duplicate testing.

7.1.4.2. If all three samples meet the specification then report the results as per the test criteria (comply or does not comply) or as given below.

for microbial limit test report the results which has maximum microbial limit count.Release the material / product for further process.

7.1.4.3. If any sample fails, then re-sampling shall be carried out to confirm the variability within the batch. Further follow the step no. 7.2 of OOS investigation phase-II.

7.2. **OOS investigation Phase-II :**

7.2.1. From phase-I laboratory investigation, when laboratory error remains unclear, a full scale OOS investigation shall be conducted after issuance of OOS investigation form (phase-II).

7.2.2. Issue the "Out of specification investigation form (Phase II)" to the analyst and make entry in the"Out of specification investigation form issuance logbook" as per the procedure given at point no. 7.1.2.4 .

7.2.3. **If assignable cause not found :**

7.2.3.1. If assignable cause not found during above investigation then re-sampling shall be carried out.

7.2.3.2. Head QC or designee shall provide justification, re-sampling quantity and sampling procedure in the "Re sampling authorization form" section of the OOS investigation form and take approval from Head Quality.

7.2.3.3. After the re-sampling authorization, Head QC or designee shall arrange for re-sampling of product as per respective SOP.

7.2.3.4. Sampling quantity and sampling position shall be depending upon the investigation and discretion of Head QC. Re-sampling shall be planned based on the risk assessment with the help of microbiologist and QA team. The sampling shall be planned from the same containers or based on the physical verification of consignment it can be planned from other additional containers (multiple container) also.

7.2.3.5. After re-sampling of material/ product Head QC or designee shall instruct analyst-1 and analyst-2 to analyse the re-sampled material/ product in duplicate testing on same day with same media preparation.

7.2.3.6. If all samples meet the specification then report the results as per the test criteria (comply or does not comply) or as given below. For microbial limit test report the results which has maximum microbial limit count.Release the material / product for further process.

If sample fails, then reject the product/material, report the initial failure results and proceed for cross functional investigation as per step no. 7.2.4.

7.2.3.7. Section head or designee shall fill the result in "Out of specification investigation form" and handover to Head QC and respective QA of QC person for reviewing and put the remark and initial / Date.

7.2.3.8. If any attachment of OOS study or other, shall be attached with the OOS form and shall be given to Head Quality for review.

7.2.4. **Cross functional investigation :**

7.2.4.1. QA of QC shall intimate QA through a written communication for cross functional investigation.

7.2.4.2. QA shall review / investigate (as per SOP of Event reporting and investigation as per SOP, but not limited to, the following in consultation with concern department:

- Evaluation of equipment cleaning records.
- Evaluation of sanitization record of PW system
- Evaluation of sampling procedure and records.
- Evaluation of any modifications done in the system.
- Evaluation of usage of the particular sampling point.
- Evaluation of sterilization details.
- Review of the results of other samples sampled & tested on the same day.
- Evaluation of the Manufacturing process of the product.
- Environmental condition (manufacturing and micro testing area)

- Evaluation of other batches for the same type of impact.

7.2.4.3. Head Quality shall make conclusion in "Final conclusion" column for the final disposition of product.

7.2.4.4. Based on the OOS investigation and it's findings corrective action / preventive action, CAPA number (if required) and root cause shall be mention under "Recommended root cause detection, corrective action and preventive action" section of OOS investigation form to control re-occurrence of the error.

7.2.4.5. Trial study can be designed for the investigation (to prove the hypothesis) purpose with concern of the Head QC/ Head QA/ Production.

7.2.4.6. Analyst shall analyse the same stage sample (for which stage trial/study planned) and fill the column of "Findings" (Results of trial/study), "Reference" (Reference of raw data), put initial in "Analysed By/ date" in "Instruction note for trial/study (attachment-3)".

7.2.5. **General procedure :**

7.2.5.1. After completion of OOS investigation and cross-functional investigation, QA of QC shall ensure for the necessary attachments filed with the OOS investigation report.

7.2.5.2. After review of OOS investigation / cross-functional investigation, a CAPA (As per respective SOP) form shall be issued by QA and ensure for compliance of the CAPA.

7.2.5.3. Impacted department head shall monitor the each recommendation.

7.2.5.4. OOS investigation shall be completed within 30 days from date of OOS result.

All failure investigation (laboratory, cross functional and re-sampling) shall be completed within 45 working days from the date of occurrence of OOS results. If closing period is exceeded then mention the reason in OOS form. But the extended period should not be more than 180 days of OOS results

7.2.5.5. In case of any confirmed failure, recall procedure as per SOP "Product recall"

Attachment-1

Out of specification investigation form(Phase-I)

Form No.	:	Issued by	:
Issued to	:	Date	:
Material/ Product :			
Batch No. :	Mfg. Date :		Exp.Date :
Stage :			
Test :	Results :		Limit :
Analyzed by :	Date :		Reference :

Details of investigation Phase-I

S. No.	Parameters	Observation	Sign / Date
1	**Reference record / document Checks :**		
1.1	Checked for specification and analytical test procedure followed.(_____)		
1.2	Check whether method is validated.		
2	**Sample Checks :**		
2.1	Sampling procedure followed (feedback based on interrogation with concern person) as per respective SOP.		
2.2	Check condition of sample and the container in which sample is stored. (Check for physical examination, storage condition, storage container, labeling, any exposure of sample etc.)		
2.3	Check condition of the sampling points.		
3	**Laboratory testing facility checks :**		
3.1	Check last qualification of testing facility (Analytical Area). Valid up to:_____		

3.2	Check environmental monitoring data of laboratory testing room on the day of analysis.		
3.3	Check cleaning data of laboratory testing room on the day of analysis.		
3.4	Check sanitization data of laboratory testing room on the day of analysis.		
3.5	Check laboratory temperature and humidity data.		
4	**Instrument/Equipment checks**		

4.1	Check balance for its calibration. Code No. of Balance : _____ Cal. Valid upto :_____		
4.2	Check any other instrument/ Equipment calibration status if used for analysis. Name of Instrument/ Equipment : Code No.:_____ Valid upto :__		
4.3	Check Laminar Air flow - Differential pressure at the time of analysis.		
4.4	Check qualification status of LAF used for analysis. LAF No. : _____ Valid upto: ___		
4.5	Check qualification status of sterilizer. Sterilizer No. : _____ Valid upto: _____		
4.6	Check qualification of incubator. Valid upto:		
4.7	Check qualification of colony counter Valid upto:		

5	**Microbiological Media checks :**		
5.1	Check media for physical appearance and for it's validity.		
5.2	Check Purity of culture.		
5.3	Check media used for analysis for validity.		
5.4	Check media sterilization record		
6	**Analyst checks :**		
6.1	Check analyst is train for the analytical technique to perform particular analysis.		
6.2	Check raw data for dilution, calculation, weighing and reading error.		
7	**Sterilization details checks :**		
7.1	Check records of media preparation Records.		
7.2	Sterilization record for require material (Thermo graph of autoclave, sterilization cycle time) used in testing.		
7.3	Sterilization of petri plate Sterilization run No.: _____		
8	**Negative Control & growth promotion test :**		
8.1	Results of negative control media plates of the same lot of media used.		
8.2	Growth promotion test record of the same lot of media used for testing.		
9	**Other checks :**		
9.1	Power failure during aseptic practices.		
9.2	Any others.		

Discussion with analyst (To identify any human error or practice deviation, if human error identify then evaluate as per attachment- 5)

S.No.	Summary of discussion	Remark of Investigator
1.	**Discussion on under standing of analytical technique:**	
2.	**Discussion on analytical process followed:**	
3.	**Discussion on sample handling during analysis:**	
4.	**Discussion on any abnormality observed during analysis:**	
5.	**Any Others:**	

Conclusion of investigation :

Remark of investigator:	Assignable cause found: Repeat the test on the same sample (or new samples) Assignable cause not found: Follow stage of OOS phase-II.
Section head : Date:	QA of QC: Date :

If assignable cause found

Repeat the test on the same sample (or new samples) by analyst-1.	
Allotted By/ Date :	Allotted To/ Date:
Results:	
Analyzed by/Date:	Reference :
Conclusion of investigation :	
Remark of investigator:	If sample passes :Release the material/product If sample fails: Analyse the same lab. Sample by Analyst-2, single time.
Section head : Date:	QA of QC: Date :
Analyse the same lab. Sample by analyst-2, single time.	
Allotted By/ Date :	Allotted To/ Date:
Results:	
Analyzed by/Date:	Reference :
Conclusion of investigation :	
Remark of investigator:	If sample passes : Analyse the same lab. sample (or new) by analyst-1, in duplicate testing.

	If sample fails: Follow OOS phase-II stage.
Section head : Date:	QA of QC: Date :
Analyse the same laboratory sample by analyst-1, in duplicate testing.	
Allotted By/ Date :	Allotted To/ Date:
Results	
Analysis-1:	Analysis-2:
Analyzed by/Date:	Reference :
Conclusion of investigation :	

Remark of investigator:	If all three sample passes : Release the material/ product. If sample fails: Follow OOS phase-II stage.
Section head : Date:	QA of QC: Date :

Justification (if Closing period exceeded)		Head QC Sign/date
Root Cause Identification :		
Final Conclusion :		

Corrective action :

(CAPA No.:_____)

Preventive action :

(CAPA No.:_____)

Head QC : Date :	Head QA: Date :

Training (If required) Date:

Purpose of training :

Training conducted by:

Topics covered :

S.No.	Name of members	Signature	Remarks of Trainees

Head QC : Date :	QA of QC: Date :

Attachment : _____

Attachment-2

Out of Specification Investigation form (Phase-II)

Form No.	:	Issued by	:
Issued to	:	Date	:
Material/ Product :			
Batch No. :			
Stage :			
Test :	Results:	Limit :	
Analyzed by :	Date :	Reference :	

OOS investigation of Phase -II : If assignable cause is not found, then re-sample the material/ product.

Re-sampling Authorization Form

Re sample the material/ product as per the respective sampling SOP.(SOP No.___)				
Justification for Re sampling:				
Batch No. / AR No.	**For investigation stage**	**Quantity**	**Authorized By/Date (Head Quality)**	**Sampled By/Date**
Analyse the re-sample material/ product in duplicate testing by analyst-1 and analyst-2 on same day using same media preparation.				
Allotted By/ Date :		Allotted To/ Date :		
Result of Analyst-1				
Analysis-1		Analysis-2		
Analyzed by/date:		Reference :		
Results of analyst-2				
Analysis-1		Analysis-2		

Analyzed by/date:	Reference :	
Conclusion of investigation:		
Remark of investigator	If all sample passes: Release the material/ product. If any sample fails: Reject the batch and proceed for cross functional investigation.	
Section head : Date:	QA of QC: Date :	
Justification (If Closing period exceeded)		Head QC Sign/date
Root Cause detection:		
Final conclusion :		
Corrective action : (CAPA No.:_____)		
Preventive action : (CAPA No.:_____)		
Training (If required) Date: Purpose of training: Training conducted by: Topics covered:		

PHARMA DEVILS

MICROBIOLOGY DEPARTMENT

STANDARD OPERATING PROCEDURE

Title: Handling of Out of Specification results for Microbiological Analysis

SOP No.:		**Department:**	Microbiology	
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S.No.	Name of members	Signature	Remarks of Trainees

Head QC: Date :	Head QA : Date :	Head-Quality : Date :

Attachment : _____

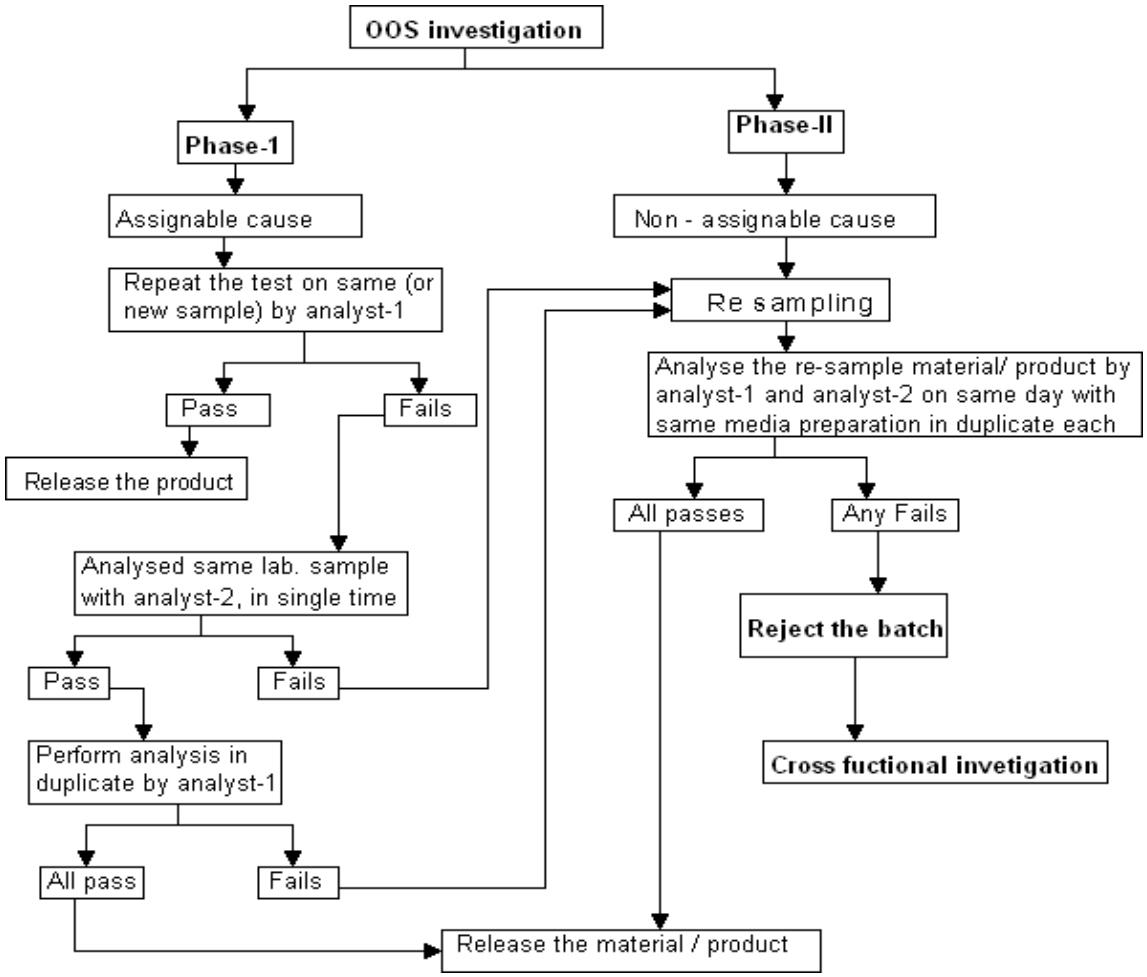
Attachment-3

Instruction Note for Trial /Study

Form No.					
Material/ Product			Batch No.		
Stage			Test :		
Results: _____			Limit : _____		
S No	**Parameters**	**Allotted By / Date**	**Findings**	**Reference**	**Analysed By / Date**
Final Conclusion:					

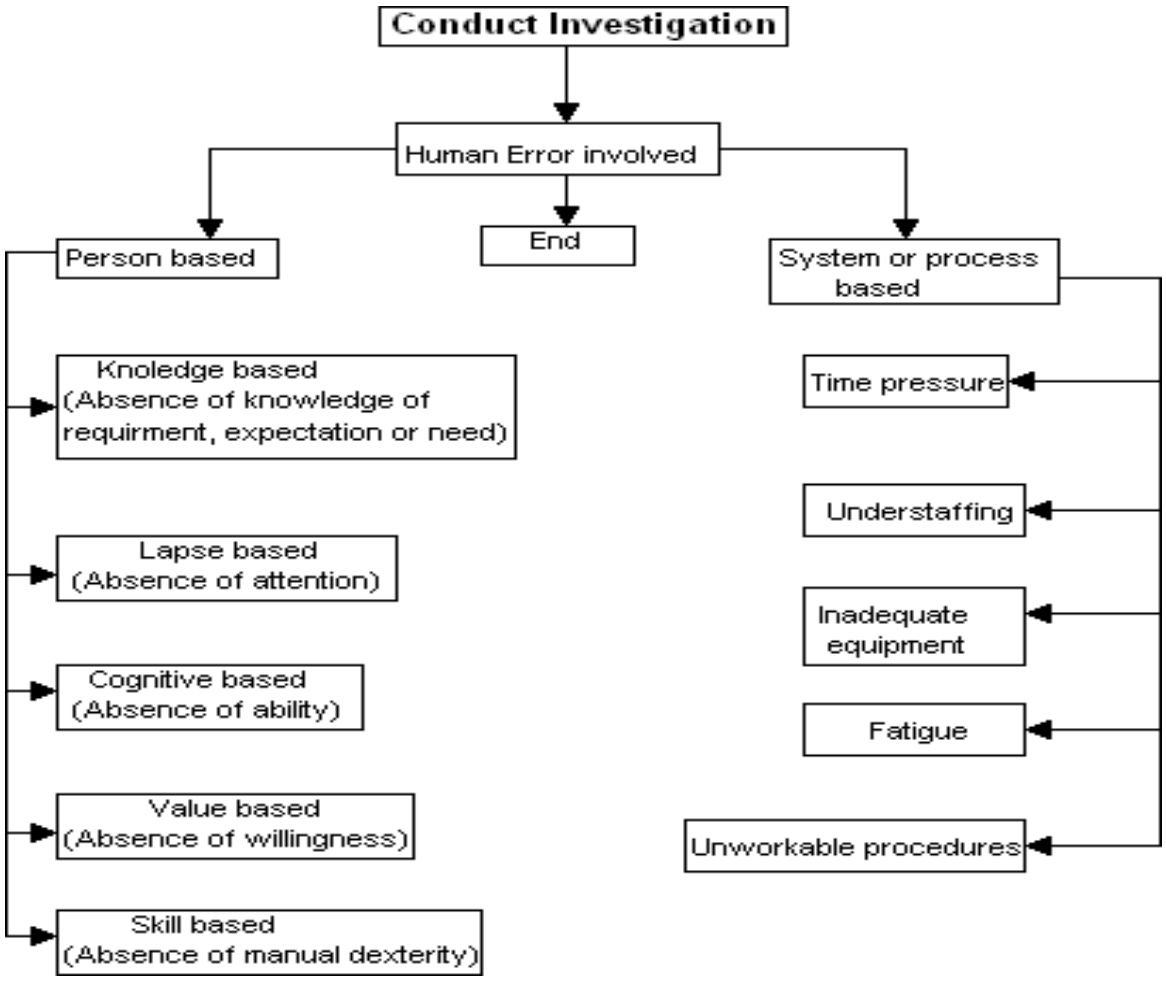
Reviewed By : Head QC			**Authorized by :** Head QA	
Date			**Date**	

Attachment-4
Flow chart for OOS investigation



Attachment-5

Flow chart for handling of human error



8. History:

Version No.		Effective Date	