1.0 Purpose

This procedure documents the steps necessary for investigating all analytical Out-of-Specification (OOS), Out-of-Trend (OOT) and unusual results, including stability results. The investigation is conducted to determine the root cause of the non-conforming result.

2.0 Scope

This procedure applies to release and stability test results that fail to meet pre-established specifications. This procedure may be applied to, but is not required for, test results that meet pre-established specifications but are out of trend with historical data or are otherwise aberrant. This procedure does not apply to results generated during non-commercial testing activities such as research and development testing, validation, and method suitability studies. This procedure does not apply to microbiological results.

3.0 Definitions

3.1 **OOS** – Out of Specification; designation which indicates that the result for the test performed does not meet the established acceptance criteria.

3.2 **OOT** – Out of Trend; designation which indicates that the result for the test performed meets specification but is not statistically consistent with historical trends.

3.3 **UR** – Unusual Result; a result that meets specification but is considered atypical, abnormal, anomalous, deviant, irregular, questionable, or unexpected.

3.4 **AR** – Associated Result; a result generated during the analysis of an OOS, OOT, and/or

unusual result that may be effected by the outcome of an investigation, but that on the surface are not suspect.

3.5 **HPLC** – High Performance Liquid Chromatography; analytical technique used to separate a mixture of compounds with the purpose of identifying and quantifying the individual components of the mixture

3.6 **INV** – an abbreviation for "Investigation" subject to this procedure and subject to formal documentation using the forms and documentations requirements of this procedure.

3.7 **Effectiveness Plan** – a defined plan that will be used to evaluate if a root cause and/ or corrective action is effective.

3.8 **Effectiveness Check** – the verification that the root cause and/ or corrective action was remediated and the quality objectives were met.

3.9 **QC** – Quality Control

3.10 **QA** – Quality Assurance

3.11 **DC** – Document Control

3.12 **GDP** – Good Documentation Practice

3.13 **GMP** – Good Manufacturing Practice

3.14 **RM** – Raw Material

3.15 **ITP** – Investigative Test Protocol

3.16 **TLC** – Thin Layer Chromatography

3.17 **FTIR** – Fourier Transform Infrared Spectroscopy

3.18 **MRB** – Material Review Board

3.19 **NCR** – Non-Conformance Report; a report documenting the details on a confirmed failure that needs further assessment by the material review board

3.20 **SME** – Subject Matter Expert

4.0 Responsibility

4.1 It is the responsibility of all QC Laboratory personnel to report INV results to laboratory management and to complete the required sections of the investigation form.

4.2 It is the responsibility of QC Laboratory Management to authorize and approve investigations.

4.3 It is the responsibility of QC Laboratory Management or designee to review the INV test, approve additional laboratory testing, and ensure completion of the investigation.

4.4 It is the responsibility of Quality Systems Management (DC) or designee to review and approve the final investigation forms and associated log for consistency.

4.5 External laboratories may be employed to investigate their results.

4.6 External laboratories may be employed for confirmation testing.

4.7 The production group will provide support for batch record reviews.

5.0 References

5.1 D-105-F1, Form, Laboratory Investigation Form

5.2 D-105-F2, Form, Investigative Test Protocol and Report

5.3 C-202, SOP, Material Review Board

5.4 QS-111, SOP, Root Cause Analysis (RCA)

5.5 QS-112, SOP, Core Quality Systems and Quality Events

5.6 QS-108, SOP, Corrective and Preventative Action (CAPA)

5.7 C-501, SOP, Document Control

5.8 C-502, Record Storage, Retention, and Destruction

5.9 Guidance for Industry, Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production – Level 2 revision, May 2022

5.10 Guidance for Industry, Statistical Techniques for Data Analysis, John Keenan Taylor, 1990 Lewis Publishers ISBN 0-87371-250-1

6.0 Overview

6.1 See SOP QS-112 Core Quality Systems and Quality Events for an overview on the core required structure for documenting and tracking quality events at Ion Nutritional Labs.

7.0 Results to Investigate

7.1 This procedure applies to Phase I, Phase II, Phase III Investigations and Investigative Test Protocols for laboratory test results that are OOS.

7.2 This procedure applies to testing associated with raw materials, manufactured bulk products, components, intermediate and finished products, and cleaning validation samples to that extent that cGMP regulations apply.

7.3 This procedure is used for OOT and/or UR tests when laboratory management agrees to investigate OOT / UR tests.

7.4 This procedure may be used to investigate results that are “Exempt” from this procedure if Quality Management decides to use this structured process.

8.0 Exemptions from Investigation

8.1 Calculation Errors – Calculation errors should be corrected by GDP.

8.2 No Established Specifications – If a specification is not in place then a result cannot be out of specification and this procedure does not apply.

8.3 Research and Development Activities

8.3.1 For data generated as a part of research and development but not associated with raw material or finished product testing for release, which includes components of validations, method's development, exploratory testing and other types of non-GMP testing, an INV is not required for an out of specification result. All results and explanations will be documented.

8.3.2 When method verification or qualification is being performed on a new formulation, all results are experimental and don't require an investigation.

8.4 Method suitability can be performed on a new product matrix to determine specificity, accuracy and precision without the requirement of an investigation when OOS results are generated. This also applies when suitability is performed by comparing the performance of multiple methods. A description of testing and data must be documented.

8.5 For qualitative assays such as TLC, FTIR, optimization testing is permitted as per respective SOP without investigation.

Examples: 1) To optimize sample / standard loading using TLC.

2) Recrystallizing sample and standard (ex. FTIR).

3) Tableting standard material (ex. FTIR).

8.5.1 Document the inconclusive conditions and the final conditions.

8.6 Alert Limits Not Met – Alert limits are not considered specifications and do not require a formal investigation under D-105.

8.7 Testing covered by protocol – If a protocol provides directions for handling results that do not meet acceptance criteria, then those directions supersede this SOP.

8.8 Staged Testing – An investigation is not required for early "stages" of testing if that stage of testing dictates next steps. For example, a USP <905> "Uniformity of Dosage Units" does not require an investigation if it fails to meet the S1 limits associated with testing of the first 10 units. The test may move on to S2 and test the additional 20 units. If test results are UR or if they would fail S2 regardless of the results of S2 additional testing, an investigation may be initiated.

8.9 Failure to meet test suitability requirements – Certain analytical methods have system suitability requirements, and results from systems not meeting system suitability requirements should not be used.

9.0 Investigation Number Assignment

9.1 DC will assign each INV an investigation number then enter the investigation into the electronic INV Log. The INV number shall be referenced on all data and attachments associated with the INV.

10.0 Data and Sample Handling

10.1 Under no circumstances may incomplete or erroneous data be deleted.

10.2 Analysts should not continue with an experiment if errors are obvious, such as the spilling of a sample solution, the incomplete transfer of a sample composite, or system suitability is not met. The analyst should document what happened and discontinue the experiment.

11.0 Investigation Forms

11.1 Laboratory investigations are documented using a single form, D-105-F1. Investigative Test Protocols use a separate form, D-105-F2 and multiple copies of the form can be used in a single investigation.

11.2 INV # - the number assigned to the investigation.

11.3 Investigation Rev # - This field should initially be "0". The revision field is used to document revisions to the investigation. This is rare, but if after the investigation is closed and the need for revision is identified, this field provides a mechanism to document the process. The previous form may be lined out using Good Documentation Practices, kept in DC and the revised form numbered sequentially by revision should be placed in the place of the lined out form.

11.4 Attachments should be placed at the end of the investigation.

11.5 DC will review the documentation for completeness and sign on the indicated forms.

12.0 Investigation Procedure

12.1 Investigation Principles / Tools described in this and referenced SOP's should be followed during the investigation process.

12.2 Upon the occurrence or discovery of an OOS result, the Chemist performing the test must notify Laboratory Management immediately.

12.2.1 Laboratory Management will evaluate unusual results prior to initiating an OOT investigation.

12.3 The analyst must immediately preserve any and all materials, chemicals, solutions, and equipment used in performing the analysis.

12.4 A preliminary evaluation must be completed by a qualified investigator to include the Phase I- Accuracy Assessment and Phase I – Confirmatory analysis prior to the expiration of any standards, samples or reagents, when possible.

12.4.1 The limited testing (re-analysis, re-injection, re-dilution, etc.) of the original test samples will be conducted when available and appropriate to confirm a laboratory error.

12.4.2 Re-analyze as per the Test Method.

12.4.3 Sample preparations that are known to be past their stability, unstable, or depleted will not be re-analyzed.

12.4.4 Data generated during re-analysis cannot be used to release material.

12.4.5 If the results for the confirmatory analysis confirm the original results obtained then further investigation is necessary in determining the root cause of the original result obtained.

12.4.6 If the results for the confirmatory analysis do not confirm the original results obtained, and if the root cause is determined to be sample preparation error or instrument error, then the original results may be invalidated. Further investigation may still be necessary in determining the root cause of the original result obtained.

12.5 If a definitive root cause is found the original test result can be invalidated with proper scientific justification. Laboratory error is the assigned cause and a new sample is prepared and tested according to the test method and documented in the conclusion of the investigation.

12.6 Laboratory management and SME's will evaluate the root cause of the laboratory error to determine whether a CAPA is required to correct and prevent the error from

reoccurring. CAPA's will be documented using QS-108, Corrective and Preventative Action (CAPA).

12.7 When there is no scientific basis for determining there was a laboratory error, a full-scale investigation is initiated upon QA or designated review of the data and confirmation of the result.

12.8 A Phase I historical review of the material will be performed to determine if there are any trends in the test results that would help determine the root cause of the OOS result. Examination of other factors such as RM inputs, new material status, and formulation accuracy can be assessed to help determine root cause.

12.9 A formal root cause analysis tool such as the 6M, 5Y, Fishbone, etc. can be used to help identify the root cause of the OOS result. Refer for SOP QS-111 Root Cause Analysis (RCA) for additional guidance.

12.10 To aid in the determination of root cause, Investigative Test Protocols (ITP)'s can be executed using D-105-F2 to generate data to confirm or refute a hypothesis.

12.10.1 Three main components are required for an ITP protocol, a hypothesis statement, a hypothesis test plan which includes an experimental design which can prove or refute the hypothesis and an outline of how to interpret the results of the data.

12.10.2 An ITP requires QA approval prior to execution.

12.10.3 The ITP results, discussion and conclusion will be documented on Page 2 of form D-105-F2, the report form.

12.10.4 The results from an ITP study cannot be used to release a product.

12.11 A Phase II Investigation is initiated after the Phase I investigation including ITP testing is complete.

12.12 A hypothesis statement is required to initiate Phase II triplicate retest of the original sample. The hypothesis statement will address the root cause of the original result.

12.12.1 If the sample is implicated as the root cause or there is insufficient sample for a triplicate retest, then a replacement sample can be used in the Phase II investigation.

12.13 Document any changes in method execution that differ from the original test conditions to support root cause determination. Changes should be within the allowed scope of the method.

12.14 Re-test will be conducted by an SME for the test being performed.

12.15 In the event that the initial OOS result is confirmed, a Phase III investigation can be initiated to evaluate potential root causes originating external to the laboratory.

12.16 The Phase III investigation uses the same format as the Phase II investigation. However, the focus of the investigation moves from the laboratory as a potential source of the OOS to all other potential sources of the OOS.

12.17 The Material Review Board should be notified and provide direction for the Phase III activities of the investigation and corrective actions, especially if the OOS involves actions from other departments.

13.0 Investigation Principles / Tools

13.1 Rules for Re-Testing:

13.1.1 Original results must be considered valid until proven to be invalid. If a re-test result generates data that does not agree with the suspect result under investigation, that fact alone does not invalidate the original result. Justification for invalidating a result must be scientifically justified and supported by investigation.

13.1.2 Re-test must have a purpose:

13.1.2.1 The number of re-tests allowed is limited because re-testing must have a purpose. For example, a re-test may be conducted to confirm the original result. If the result is confirmed under the retest plan, it does not make sense to try to confirm the result again.

13.1.2.2 It is permissible to re-test under protocol using an ITP to collect evidence to support a theory. The ITP plan must define the purpose of the experiment and define the criteria by which the theory would be proven or disproved.

13.1.2.3 For example, a protocol written to test a theory about the validity of original results of product to be tested for release may generate re-test data as part of that investigation. This data may be defined in the protocol to only be used to test that theory. Regardless of whether the re-test results meet required specifications or not, they cannot be used to release the product if defined this way. The results would be used to either prove or disprove the theory.

13.1.3 Appropriate samples must be used: The sample used for the re-testing or investigative testing should be taken from the same homogeneous material that was initially tested and yielded the suspect result. For example, the original liquid sample solution that was injected into the chromatographic system may be used, while a solid sample may require an additional weighing from the same sample composite that had been prepared by the analyst. If a sample cannot be taken from the same homogeneous material that was initially tested, then the re-test would also be considered re-sampling. The rules for re-sampling would apply.

13.2 Rules for Re-Sampling:

13.2.1 Re-sampling should be avoided if at all possible. The suspect result was generated

from the original sample, and that sample may be the cause of the result. Re-sampling unnecessarily could hide the true cause of the result and impair the investigation. This is especially true for content uniformity or blend uniformity samples where the purpose of the test is to look for variability of the samples. The following criteria must be met when re-sampling is used:

13.2.1.1 Re-testing criteria apply: Since re-sampling will require re-testing to generate data, the rules for re-testing also apply to re-sampling with the exception of the sample used.

13.2.1.2 Invalid Sample: It is appropriate to re-sample if it can be shown that the original sample was obtained, stored, or handled improperly in such a way as to cause the sample integrity to be compromised. If this can be proven, it will invalidate not only the sample, but also the original suspect result. Re-sampling may be necessary to prove or disprove a theory about the validity of the original sample.

13.2.1.3 Shortage of sample: It may be appropriate to re-sample if there is not enough sample to complete an investigation. If the original sample expires or is too small to continue an investigation, re-sampling will be necessary. This does not negate the need to evaluate the impact that re-sampling may have on the result.

13.2.1.4 Impact must be considered: The impact that re-sampling may have on the ability of the investigation to identify the true cause must be considered. If re-sampling has occurred, it must be able to be defended in the scientific evaluation of the suspect result.

13.3 Reportable Results:

13.3.1 If a laboratory error was discovered in the investigation, the results associated with the error must be invalidated and followed with valid results as directed in

the investigation protocols.

13.3.2 If no error could be identified in the original test results, and there is not scientific basis for invalidating the results under investigation, then the test result being investigated must be reported.

13.4 Averaging:

13.4.1 Averaging, though it can be a rational and valid approach, generally is a practice that should be avoided. This is because averages hide the variability among individual test results. The phenomenon is particularly troubling if testing generates both OOS and passing individual results which, when averaged, yields a result that is within specification. Here, relying on the average calculated figure without examining and explaining the individual OOS results through an investigation process is highly misleading and unacceptable.

13.4.2 Dosage form content uniformity results or powder blend/mixture uniformity never should be averaged to obtain a passing uniformity value. Nor should equipment cleaning validation/verification results from multiple swab sites be averaged. In the former case the tests are intended to measure variability; in the latter case it may obscure a localized high level of impurity in a specific swab area.

13.4.3 It should be noted that a test might consist of replicates to arrive at a result. For instance, an HPLC assay result may be determined from the average value of the peak responses from a number of consecutive replicate injections from the same sample preparation. The assay result for each sample would be calculated using the peak response average. This determination is considered one test and one result. This is a distinct difference from the analysis of different portions from a lot, intended to determine variability within the lot.

13.5 Outlier Test- Criteria

13.5.1 An outlier is a value in a data set which appears to deviate markedly from other members of the same sample in which it occurs, and has low statistical probability of belonging to the same population. Outlier's tests can indicate a test error when used with re-measurement or retesting of samples that are expected to be homogenous (i.e. crushed tablets, solutions). Under no circumstances may an outlier test be used to disqualify a test where uniformity is being tested (i.e. content uniformity and dissolution).

13.5.2 The preferred method for outlier analysis is the Q-Test.

13.5.2.1 Rank the data points in order of increasing value X1 < X2 <…Xn.

13.5.2.2 Decide whether X1 or Xn is suspect.

13.5.2.3 Calculate the sample average (A) and standard deviation (s) using all of the data for the moment.

13.5.2.4 Calculate Q as follows using equation 1 if X1 is suspect or equation 2 if Xn is suspect:

$$Q_{exp} = \frac{X_2 - X_1}{X_N - X_1} \qquad Q_{exp} = \frac{X_N - X_{N-1}}{X_N - X_1}$$

13.5.2.5 90% is the accepted confidence level for risk of false rejection.

N	Qcrit (CL: 90%)	Qcrit (CL: 95%)	Qcrit (CL: 99%)
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

13.5.2.6 If the calculated value exceeds the Qcrit value, the data point is a statistical outlier; otherwise the data is not a statistical outlier.

13.5.2.7 If the original data does not contain a statistical outlier or combined initial and secondary data does not contain a statistical outlier based on the Q-Test, the raw original data will be combined with the secondary test data then averaged. The averaged result will be the reported value.

14.0 Documentation Requirements

14.1 All documentation will be distributed and maintained as outlined in SOP C-501 Document Control and SOP C-502 Record Storage, Retention, and Destruction.

15.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	03/04/10	New	-	-
1	10/18/12	Clarified and reorganized SOP, changed format, created OOS log and revised OOS form	-	-
2	07/08/13	Changed "QC Manager" to "Lab Manager", changed in section 5.1.4 & 5.1.4.2 "QC Analyst" to "DC", section 5.5.5 "QC Laboratory" to "DC office", changes in section 5.4.6.6, 5.5.6. Clarified process for determining outlier using Q Test.	13-593	B. Johns
3	03/25/14	Added Out of Trend Investigation criteria. Added a new section on Data Handling. Clarified form data entry. Added new section of exploratory testing for root cause. Changed minimum signature requirements for test approval. Phase II investigation controlled by QA / Production. Expanded corrective action responsibilities. Changed outlier criteria to 90% confidence level. Changed form D-105-F1 to include Exploratory Testing section, adjusted format, reduced signature requirements.	14-0277	B. Johns
4	01/13/16	Removed paper log; expanded responsibilities	16-0030	D. Popp
5	08/01/17	Added impact assessment requirements during investigation.	17-0762	S. Millar
6	12/29/18	Complete rewrite.	18-0450	D. Herd
7	09/03/19	Added reference to QS-112 Quality Events SOP.	19-0605	A. Roxbury
8	03/28/22	Added requirements for short-term and long-term monitoring of effectiveness check.	CC-22-0140	J. Sassman
9	06/13/24	Reduced investigation forms from ten to two, form 1 for investigation, form2 for investigation test protocols. Restructured investigation process to three Phases, Phase I- Preliminary investigation, Phase II – retest to challenge laboratory issue, Phase III- retest to challenge non-laboratory root causes. Made CAPA structure external using SOP QS-108. Added additional detail to improve clarity of instruction. Added the use of a root cause analysis tool for determining root cause, referencing SOP QS-111. Updated flow chart to reflect new process	CC-24-0278	B. Johns

16.0 Attachments

16.1 Attachment 1 - Lab Investigation Flow Chart

Attachment 1 – Lab Investigation Flow Chart