Centre for Biopharmaceutical Excellence

Good Laboratory Practices for Analytical Laboratories

Module Topics

- Regulatory Guidelines
- Quality Management System
- Key G(QC)LP Requirements
- Computerised Equipment

Some Useful Reference Documents

- EU/PICs Guides to Good Manufacturing Practices - Sec. 6 Quality Control
- WHO good practices for pharmaceutical quality control laboratories- TRS 957
- WHO good practices for pharmaceutical microbiology laboratories- TRS 961
- WHO GMP for Biological Products Draft 18 2015
- EU/PICs Guide - Annex 8 - Sampling of Starting Materials)
- Guide 17025 General Requirements for the competence of calibration and testing laboratories.
- British Pharmacopoeia (BP) and European Pharmacopoeia (EP) and United States Pharmacopoeia (USP)
- USA Code of Federal Regulations CFR 21 Part 211; Subparts 160 and 194
- FDA Guidance
  - Inspection of Pharmaceutical Laboratories (1993)
  - Analytical Method Validation & Chromatographic Methods
  - Handling OOS Conditions
- ISO 17025 International Standard for Laboratory Quality Systems
- WHO TRS 996 ANNEX05 (Data & Record Management)

WHO Guidance's

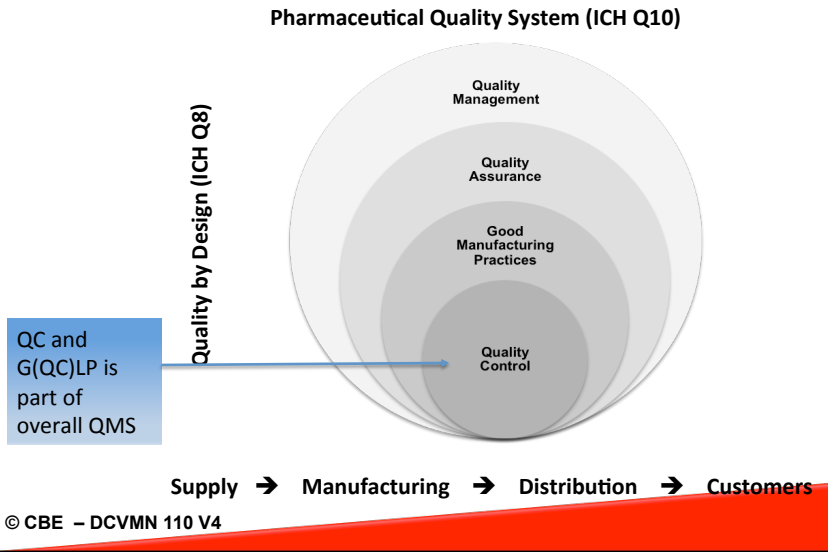
- The following WHO guidance's exist and are in line with PIC, FDA and ISO 17025.
  - WHO good practices for pharmaceutical quality control laboratories- TRS 957
  - WHO good practices for pharmaceutical microbiology laboratories- TRS 961
  - WHO GMP for Biological Products Draft 18 2015

EU/PICs cGMP
Chapter 6 Quality Control

PRINCIPLE

- Quality Control is concerned with sampling, specifications and testing as well as the organisation, documentation and release procedures which ensure that the necessary and relevant tests are carried out, and that materials are not released for use, nor products released for sale or supply, until their quality has been judged satisfactory.
- Quality Control is not confined to laboratory operations, but must be involved in all decisions which may concern the quality of the product.
- The independence of Quality Control from Production is considered fundamental to the satisfactory operation of Quality Control.

Pharmaceutical Quality System, Quality Assurance, GMP and Quality Control



Key Minimum Laboratory SOPs

Management/Infrastructure

- Organization and management
- Quality management system
- Control of documentation
- Control of Records
- Data-processing and checking
- Computerized Laboratory Systems
- Personnel and Training
- Premises
- Equipment, instruments and other devices
- Contracts

Materials/Equipment/Devices

- Reagent Preparation
- Control of Reference substances and reference materials
- Calibration and maintenance of equipment
- Qualification of equipment instruments and other devices.
- Traceability

Key Minimum SOPs

Working Procedures

- Incoming samples
- Analytical worksheet
- Validation of analytical procedures
- Testing
- Evaluation of test results
- Release of results and Certificate of analysis
- Retained samples
- General rules codes of conduct
- Laboratory Safety/housekeeping

Microbiology- additional

- Environmental monitoring in the laboratory
- Cleaning, disinfection and hygiene
- Sterility test facilities
- Reagents and Media
- Organism Resuscitation
- International stds and ref cultures
- Sampling, sample handling and identification
- Internal QC and controls
- Validation of Microbiological Methods

Specific Elements of QC G(QC)LP

- **Test Methods and Test Reports**
- **Lab books/sheets Instrument Records, and Calculations**
- **Conditions of tests and instrument settings**
- **Test Methods Validation Protocols, data and reports**
- **Other Records and Data**
  - Testing and standardisation of reference standards, reagents and standard solutions.
  - Calibration of laboratory instruments.
  - Instrument Logs.
  - Records of all stability testing performed.
  - Investigations of OOS conditions.
  - Certificates of Analysis from Suppliers.

Specific Rules for Laboratories CFR Sec. 211.194 Laboratory Records

.(a) Laboratory records shall include complete data derived from all tests necessary to assure compliance with established specifications and standards, including examinations and assays, as follows:

(1) A description of the sample received for testing with identification of source (that is, location from where sample was obtained), quantity, lot number or other distinctive code, date sample was taken, and date sample was received for testing.

(2) A statement of each method used in the testing of the sample. The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability (3) A statement of the weight or measure of sample used for each test, where appropriate.

(4) A complete record of all data secured in the course of each test, including all graphs, charts, and spectra from laboratory instrumentation, properly identified to show the specific component, drug product container, closure, in-process material, or drug product, and lot tested.

(5) A record of all calculations performed in connection with the test, including units of measure, conversion factors, and equivalency factors.

(6) A statement of the results of tests and how the results compare with established standards

(7) The initials or signature of the person who performs each test and the date(s) the tests were performed.

(8) The initials or signature of a second person showing that the original records have been reviewed for accuracy, completeness, and compliance with established standards.

(b) Complete records shall be maintained of any modification of an established method employed in testing. Such records shall include the reason for the modification and data to verify that the modification produced results that are at least as accurate and reliable for the material being tested as the established method.

What are G(QC)LP compliant laboratory records

FDA CFR 211 - Sec. 211.194

6) Results of tests and how the results compare with established standards of identity, strength, quality, and purity.

(7) The initials or signature of the person who performs each test and the date(s) the tests were performed.

(8) The initials or signature of a second person showing review for accuracy, completeness, and compliance

(b) Complete records shall be maintained of any modification of an established method employed in testing.

(c) Complete records shall be maintained of any testing and standardization of laboratory reference standards, reagents, and standard solutions.

(d) Complete records shall be maintained of the periodic calibration of laboratory instruments and recording devices

(e) Complete records shall be maintained of all stability testing performed in accordance with Sec. 211.166.

G(QC)LP Audit Deficiencies – Data Recording and Review

- Original chromatographic data not recorded in lab books - not available for inspection.
- Stability data notebooks not signed by reviewer.
- Lab worksheets do not allow record method performed and calculations used.
- SOP does not specify worksheet ID of each lab instrument used in analysis.
- No provision for maintaining hard copy printouts of absorbance values used in calculation of finished product assay results.
- No record of identity of equipment used to perform finished product testing.

G(QC)LP Audit Deficiencies – Data Recording and Review

- Records not maintained of lot numbers and USP status of standards used for product testing or equipment calibration.
- Failure to identify different equipment, test methods, and testing facilities from those submitted in the Application
- Certain lab reports omitted from analyst notebooks..
- Raw data for HPLC assays was deleted from computer system and backup tapes were not maintained..
- Dates on content uniformity computer report different from lab notebook.
- HPLC chromatogram, computer report and analytical procedure do not contain identifying
- Number allowing for reference to lab notebook.

Sampling

- There should be a written sampling protocol for each starting material, in- process bulk and finished product. The sampling plans should be based on sound statistical principles and carried out in such a manner as to preclude bias.
- EU/PICs cGMP Annex 8 - Sampling of Starting and Packaging Materials:
  - The identity of a complete batch of starting materials can normally only be ensured if individual samples are taken from all the containers and an identity test performed on each sample.
  - It is permissible to sample only a proportion of the containers where a validated procedure has been established to ensure that no single container of starting material has been incorrectly labelled.
- Sampling plans are used which approximate to $\sqrt{n}+1$, such as ANSI/ASQ Z1.4 and ISO 2859-1: Sampling by Attributes.

Sampling Plans

- Published sampling plans e.g. ANSI/ASQC Z1.4, ISO 2859, BS6001 (attributes) and ANSI/ASQC Z1.9, ISO 3951, BS6002. (Variables)
- Publish written sampling procedures that describe:
  - the method of sampling and environmental conditions
  - the number, location and amount of sample
  - the sampling equipment
  - instructions as to the sub-division or pooling of the sample
  - the identification procedure for sample containers
  - the sample storage conditions
  - any safety precautions required.

Some Important Principles for Chemical Starting Materials Control

- Establish and use a first in first out (FIFO) system for materials and products (using MRP if possible).
- Limit pooling of assay samples to n = 5. No pooling of identity samples
- Periodically review the status of materials or products, should their storage be prolonged to a period which may cause failure to comply with the relevant quality control specifications.
- A standard procedure for re-examination of starting materials should be written; the procedure should include retest strategy (particularly for possible labile materials)
  - Again use of MRP to trigger resampling and testing in a timely manner is useful

Identity Testing of Finished Product

- Where the manufacturer makes other products which are clearly distinguishable from this product by visual examination, the identification test may be carried out on a sample of the bulk final product.
- Where different products are not clearly distinguishable the test should be carried out on a sample of the packaged product.
- The identity test should be definitive for identity of the active(s) and strength.
- USP/EU/SFDA recognise NIR/RAMAN as identification methods when backed with solid validation package.

Laboratory Standards/Reagents

Laboratory Standards

- SOP for Standards Management
  - selection, standardisation, change, and control.
  - Register, Logging and Inventory System.
  - Labelled.
  - Date of introduction and expiry.
  - Complete description (name, source, Lot number).
  - Strength, activity and confidence interval.
  - Storage conditions
  - Protected (heat, light, humidity, irradiation, vibration)
  - Standard Lot Number must appear in testing record.
  - Usage “as is” or “anhydrous basis” etc.

Chemicals/Reagents

- Follow Compendia- freshly prepared etc.
- Inventory with received, opened and expiry dates.
- Standardisation records.
- Complete labelling:
  - Standard Name
  - Batch Number (some companies assign internal number)
  - Date of preparation
  - Date of expiry
  - Storage conditions
  - Strength

G(QC)LP Audit Deficiencies – Reference Standards

- Accuracy study done using a standard with low purity.
- Procedures provides for standard solutions to be held and used for six months or more, not prepared fresh for each analysis.
- HPLC chromatograms for standards show changes in peak base-line with no comment, adjustment or investigation.
- No appropriate purity/stability tests on non-USP reference std.
- Standards stored in desiccator whose silica gel was expired, absorbed moisture, used on anhydrous basis.
- Failure to assay and/or maintain records of analysis of reference standards.
- Failure to conduct cross over verification of new primary and existing secondary standards- step change in stability studies.

G(QC)LP Audit Déficiences – Instrument Qualification / Calibration

- No calibration of HPLC units, UV spectrophotometer, IR Spec, and pH meter.
- Calibration of auto-pipettes was out of date.
- No records of full calibration of analytical balance just daily checks and not covering full range.
- Incubators had no record of temperature mapping nor daily monitoring checking.
- Calibration SOP does not require limits for accuracy and precision.
- Calibration of QC pH meter and production in-process meter not identical and standards not maintained- no assigned responsibility for production meter.
- Freezers at -20C and -80C not calibrated since installation 4 years ago- heavily iced up.

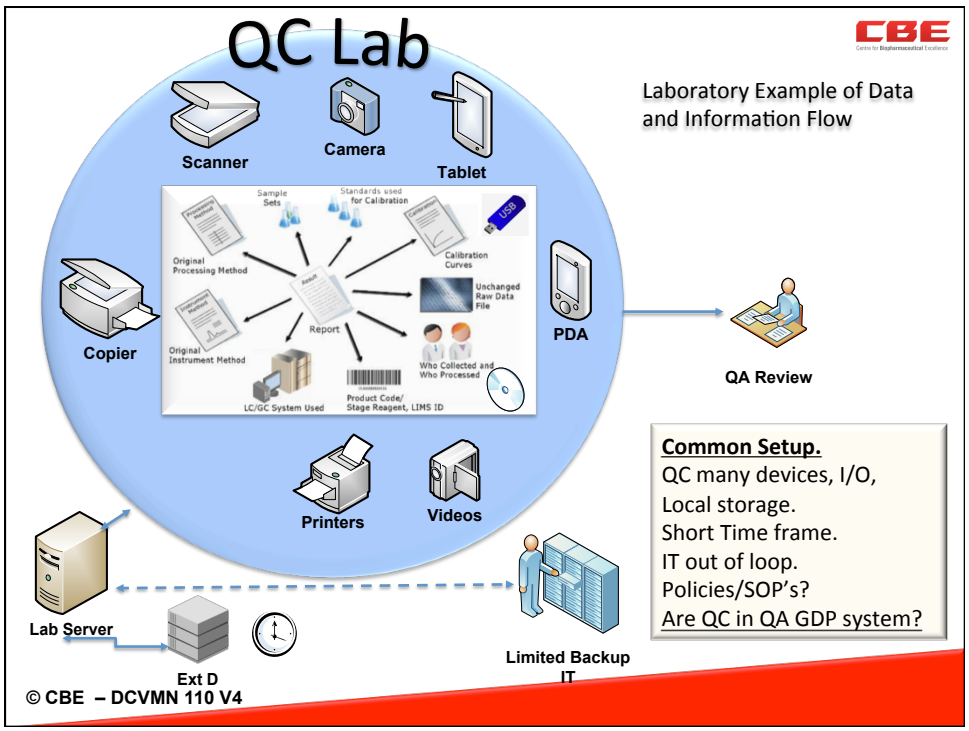
G(QC)LP Audit Deficiencies – Instrument Qualification / Calibration

- Spectrophotometers used to assay finished product not removed from service after failing wavelength accuracy portion of calibration. No Id system for OOcalibration.
- Calibration procedures not evaluated for wavelength accuracy in the range used.
- Assay test software system not validated, lacks security system and backup, and does not provide hard copy to allow verification of instrument parameters.
- Columns not tested or conditioned prior to use, no written guidance for replacing columns and no records of tests and calibration following maintenance.
- HPLC system components regularly moved/interchanged without any additional checks or impact assessment although each unit regarded as a "set" via equipment numbering.

Computers In the Laboratory- Introduction to Data Integrity

Computerised Systems and G(QC)LP (some useful standards)

- EU/PICs Code of GMP - Annex 11
- PICs (Draft) Good Practices for Data Management and Data Integrity in GMP/GDP Environments
- MHRA GxP - Data Integrity Definitions and Guidance – Draft July 2016
- FDA Data Integrity and Compliance With CGMP Guidance for Industry – April 2016
- US CGMP Compliance Policy Guides for Computerized Drug Processing:
  - # 7132a.07 Input/output checking
  - #7132a.08 Identification of "Persons" on batch Production and Control Records
  - #7132a.11 CGMP Applicability to Hardware and Software
  - #7132a.12 Vendor Responsibility
  - #7132a.15 Source Code for Process Control Application Programs
- FDA Guide to Inspection of Computerized Systems in Drug Processing.



CSV in QC

- The basic requirement's of chapter 11 apply and should follow the GAMP categorisation.
- Initial Impact assessments should be carried out to determine the GAMP category and level of validation required. (See CSV and Risk Assessments presentation).
- Basically highly configurable systems such as LIMS and Chromatographic integration systems would be GAMP category 4 and require FS/URS/IQ/OQ/PQ.

Key Principles Restricted Access

- User access controls, both physical and electronic, shall be configured and enforced to prohibit unauthorised access to, changes to and deletion of data. For example:
  - Individual Login IDs and passwords should be assigned for all staff needing to access. Shared login credentials do not allow for traceability to the individual who performed the activity. **For this reason, shared passwords, even for reasons of financial savings, must be prohibited.**
  - Input of data and changes to computerised records must be made only by authorised personnel. Companies should maintain a list of authorised individuals and their access levels.
  - Admin access should be strictly controlled.
  - Admin staff should be independent from the tasks i.e. QC supervisor/ QA Officer. Or have different log ins for non admin duties.

Key Principles - Backup/Archiving

Backups

- Storage of data must include the entire original data and metadata, including audit trails, using a secure and validated process.
- If the data is backed up, or copies made, then they must also have the same levels of controls to prohibit unauthorised, changes to and deletion, alteration of data.
- I.e. a back up of data onto portable hard drives must prohibit the ability to delete data from the hard drive.
- True copies of dynamic electronic records can be made, provided that the entire content (i.e., all data and metadata is included) and meaning of the original records are preserved.
- Software needs to be kept current to review such record.
- Backups should be stored offsite typically on a daily basis i.e. QC manager keeps QC backup.

Archiving

- The record retention procedures should include data and metadata.
- The same records and data that are backed up should be archived according to policy.
- The archives must remain readable through system/software updates.
- Archived data restoration should be periodically tested according to SOP's.
- The archives should be in secure and environmentally controlled and restorable after disaster.
- The archives should be managed such that data migration to another system if required can occur.
- There should be the facility to produce meaningful archive reports of content.
- There should be procedure linked to paper record destruction timeframes based upon regulatory requirements.

Data Integrity Landscape In the Laboratory

What will be covered

- FDA Warning Letter Examples
- Laboratory Examples
- Specific Rules for Laboratories
- Assessing DI Vulnerability

Recent Findings Concerning Personnel

US Warning Letter 320-14-08:	• Five completed preventive maintenance forms were torn. • A staff member stated that he mistakenly tore and destroyed these original records.
US Warning Letter 320-14-13:	• The inspection revealed that your firm falsified documents designed to demonstrate the effectiveness of CGMP training. • Your production head admitted to pre-filling out the answers to post-training comprehension assessment questions and entering the names of employees on these documents.

Recent FDA Warn Letter Example

- Your firm's Computer system for entering test results and storing certificates of analysis (CoA), which document whether a drug meets specifications, does not have sufficient controls to prevent unauthorized changes to a CoA after quality unit approval.
- During the inspection, our investigator reviewed CoA stored on computer #16, all of which were approved by the quality unit.
  - A manager demonstrated for our investigator how results on an already finalized CoA could be manipulated after the formal quality unit approval.
  - Also, the quality unit's electronic signatures on these CoA were uncontrolled images of signatures rather than certificate-based electronic signatures.

Error, Falsification or Compliance ?

US Warning Letter 320-15-06

- In correspondence with the Agency, you indicate that no malicious data integrity patterns and practices were found.
- Also, you state that no intentional activity to disguise, misrepresent, or replace failing data with passing data was identified and no evidence of file deletion or manipulation was found.
- **Your response and comments focus primarily on the issue of intent and do not adequately address the seriousness of the CGMP violations found during the inspection.**

Examples of Falsification

Operator level: WL 320-14-01:

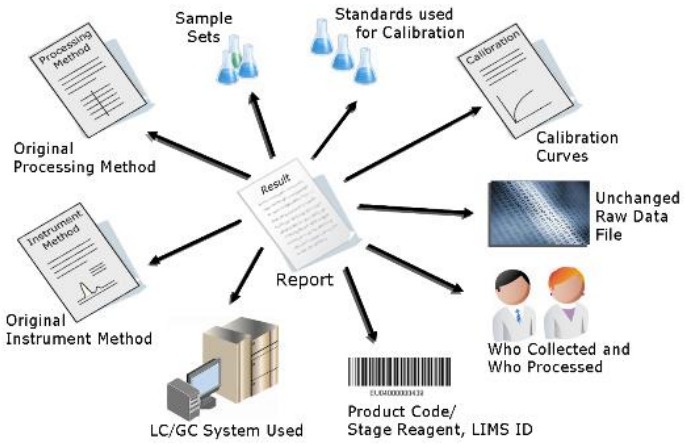
- your operator stated that he records the two weights with xxx significant figures into the batch record **from memory**….

- **Management: WL 320-15-12**
- the Technical Director backdated his own signature to the date the quality unit (QU) reviewed and released your drug product.

Control of GMP Records and Forms

- Falsification can originate from uncontrolled user access to documents (records, forms and logs)
- PICs Draft Guidance and FDA expect that access to forms and blank records is restricted;
- Historically access was driven by convenience;
  - Now requires QA oversight;
- Significant challenge to industry;
  - 100% electronic records – requires massive change/project
  - Hybrid record system (combination of Paper and eRecord)
  - Paper based issue of forms and records, as needed

Data Integrity in the Laboratory



Laboratory Data Integrity Challenges

- Focus of some Inspectors now
- FDA CFR 211.194 has a full list of compliance requirements for QC Laboratories.
- It covers:
  - *A complete record of all data secured in the course of each test, including all graphs, charts, and spectra from laboratory instrumentation, properly identified to show the specific component ……, or drug product, and lot tested*
- The above is overlayed by the Part 11 / ERES requirements;
- Electronic data must have an audit trail (or equivalent systems);
- Lab complexity increased by instrument data manipulation, automatic data capture, use of spread sheets and LIMS.

Specific Rules for Laboratories
CFR Sec. 211.194 Laboratory Records

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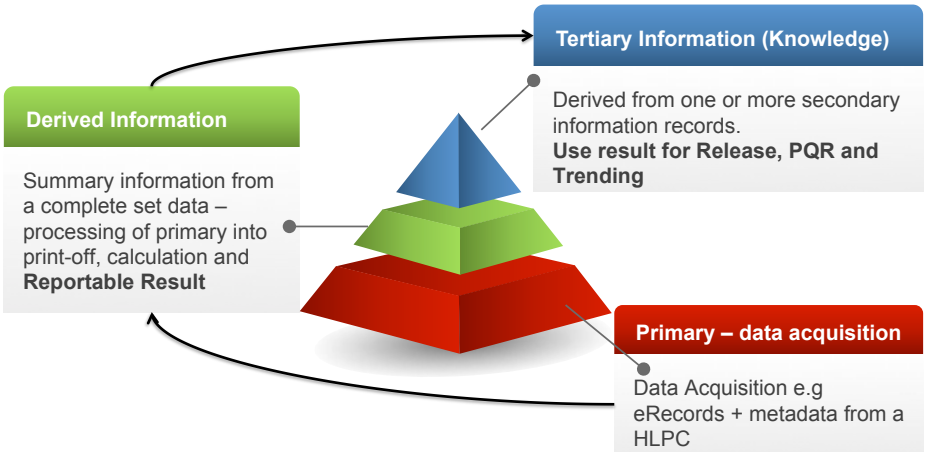
Converting Laboratory Data to Information, then Knowledge



GDocP and GDRP

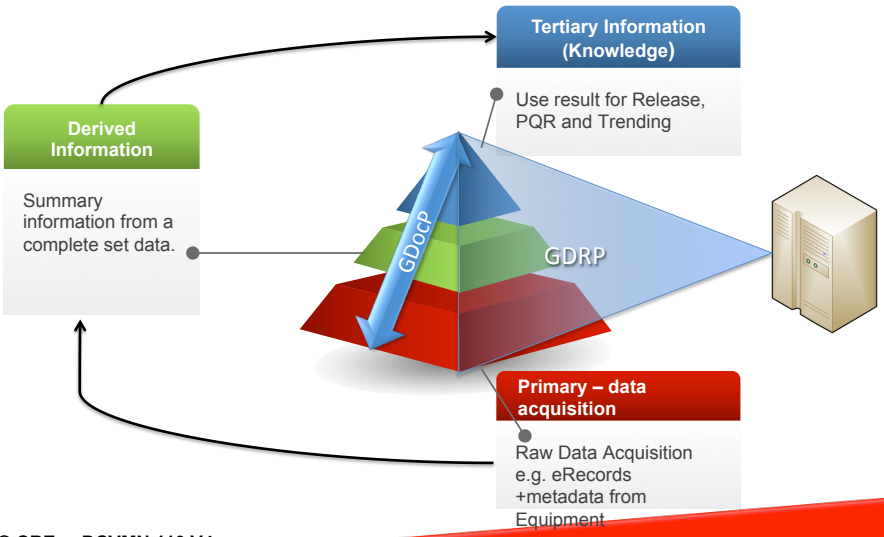
WHO TRS 996 ANNEX05 (Data & Record Management)

- **GDocP refers to:**
  - *"Good documentation practices, are those measures that collectively and individually ensure documentation, whether paper or electronic, is secure, attributable, legible, traceable, permanent, contemporaneously recorded, original and accurate."*

- **GDRP (Good Data and Record management Practice) refers to:**
  - *"The totality of organized measures, that should be in place to collectively and individually ensure, that data and records are secure, attributable, legible, traceable, permanent, contemporaneously recorded, original and accurate, and that if not robustly implemented, can impact on data reliability and completeness, and undermine the robustness of decision-making based upon those data records."*

*http://www.who.int/medicines/publications/pharmprep/WHO_TRS_996_annex05.pdf

How GDP/GDRP and Data Integrity Interact

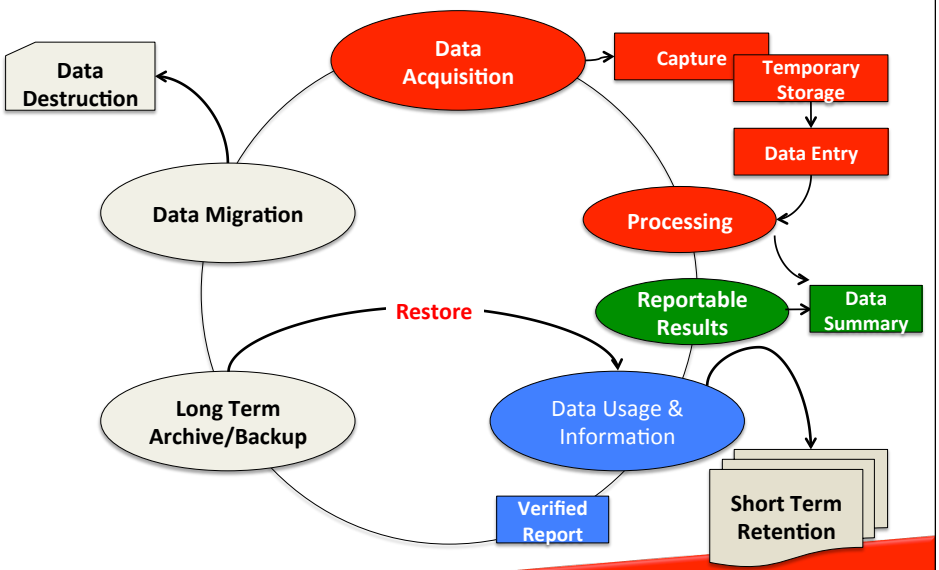


Key Data Integrity Attributes – ALCOA+

Attributable	Legible	Contemporaneous	Original	Accurate
• Who actually acquired the data or performed the actions and when? • Signed and dated	• The data must be legible / readable. • The record should be permanent • The record should be enduring and be on proven storage media	• Data must be recorded in real time as and when it occurred. • Should be carried out in close proximity to its occurrence.	• Data must be preserved in its unaltered state. • If raw data is not kept there must be solid documented justification. • The records should not have been tampered with.	• Data must correctly reflect the measurement or observation • There should be no omissions.

+ adds Complete, Consistent, Enduring and Available

ALCOA and Data Integrity "Lifecycle"



Laboratory DI Issues
(US Warning Letter 320-16-07)

- The entries for July 10 –13, 2014, were not present when the investigator initially reviewed the log. When questioned by the investigator, the laboratory analyst responsible for performing these entries stated three times that she had documented the newly-completed temperature values at the time of performance.
- The same analyst's supervisor later admitted to directing the analyst to fill out the logbook after the fact.

Laboratory DI Issues
(US Warning Letter 320-16-31)

- Failure to have laboratory control records that include complete data derived from all laboratory tests conducted to ensure compliance with established specifications and standards……..
- Prior to conducting official analyses, your laboratory performed **"experimental" analyses** on product batches to assess whether your API met specifications, but failed to document these "experimental" tests in official laboratory records or to justify their exclusion. Our investigator found the results of 2,404 (HPLC) injections in a folder titled "Experimental" on instrument SZG-002-006I.
- Your management provided different explanations in an attempt to justify the practice, including "fear" that the sample results would not pass.

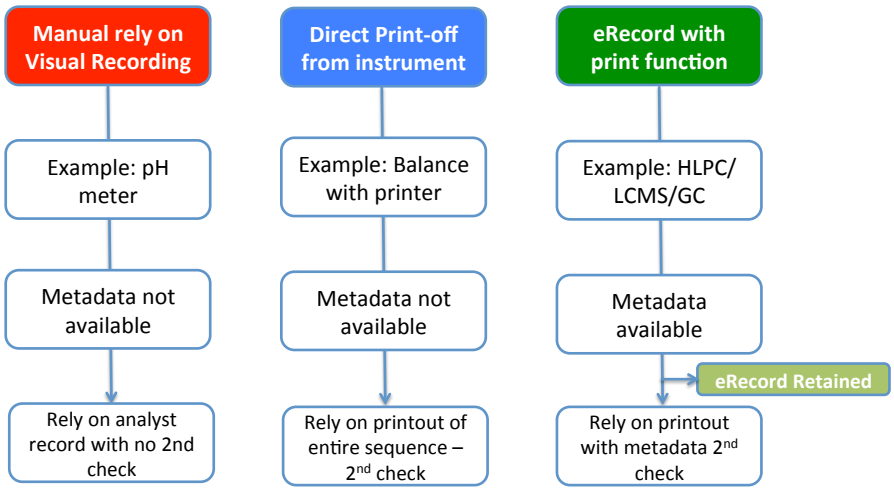
Laboratory DI Issues
(US Warning Letter 320-16-31)

- During the inspection, your firm provided our investigator a chromatogram for an assay analysis of (b)(4) batch (b) (4) dated August 30, 2014, at 9:46:39 a.m. Your firm later submitted to FDA a different chromatogram corresponding to the same analysis, instrument, date, time, and batch.
- The second chromatogram appears exactly the same as the one provided during the inspection, but it includes a different method file name, column type and serial number, and system temperature.
- Both versions of these documents cannot represent the actual assay analysis that you conducted for batch (b)(4) on August 30, 2014, at 9:46:39 a.m.

Laboratory DI Issues
(PICs/ TGA 2016)

- The current laboratory data acquisition software lacks an audit trail function, which is a GMP requirements as below:
- *Annex 11 – Clause 9 - Audit Trails - Consideration should be given, based on a risk assessment, to building into the system the creation of a record of all GMP-relevant changes and deletions (a system generated "audit trail"). For change or deletion of GMP-relevant data the reason should be documented. Audit trails need to be available and convertible to a generally intelligible form and regularly reviewed.*
- The sole system administrator for all functions, including calculations, integration algorithms and data back-up was the QC Manager of the Laboratory, not a person independent of the laboratory.

Laboratory Raw Data Collection



Laboratory Data Generation and DI Challenges

Method →→	Lab Notebook Observation	Simple Instrument	Balance Printer	Spectrophotometer	HPLC/GC	Lab. Data Acquisition System	LIMS System
GAMP Class	NA	Cat. 2	Cat. 2	Cat. 3	Cat. 4	Cat. 4	Cat. 4 or 5
USP<1058>	N/A	A	B	C	C	N/A	N/A
Recording Mode	Manual	Manual	Printout	Printout & erecord	Printout & erecord	Printout & erecord	Printout & erecord
Metadata	No	No	Maybe	Yes	Yes	Yes	Yes
Raw Data	Manually Written	Manually Written	Printout	eRecord	eRecord	eRecord	eRecord
DI Challenges	No independent check	No independent check	Limited printout	Printouts not raw data. Metadata Key	Printouts not raw data. Metadata Key	Printouts not raw data. Metadata Key	Printouts not raw data. Metadata Key
Recommended DI Controls							

DI Example: Analytical Balance

Options

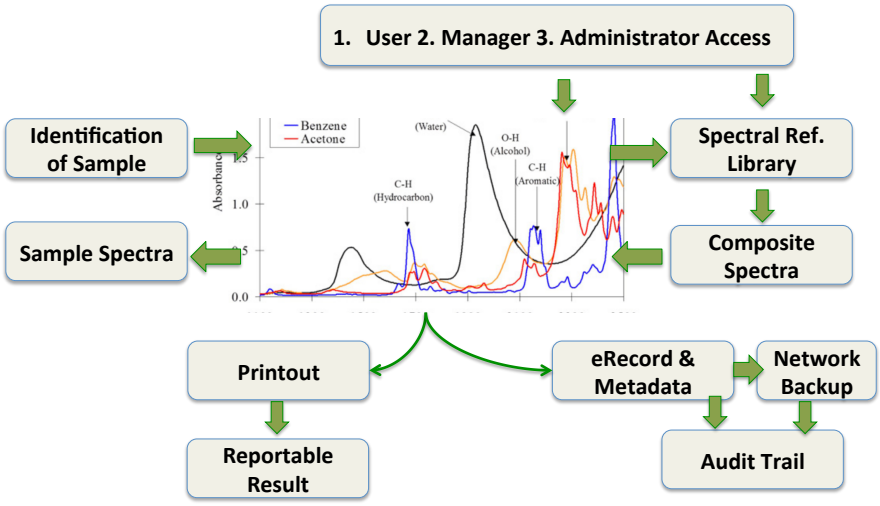
1. No printer – record data in lab. book**
2. Standalone Printer – automated data capture
3. Interface to LIMS or Electronic Note Book

** Today most balances expected to have printouts

Challenges and Features:

- Who can access the printer clock ?
- Can access levels be restricted ? Authorised users
- What data needs to be printed ? Whole sequence
- Controls if linked to a data capture system such as LIMS
- Ability to barcode read and reading integrity

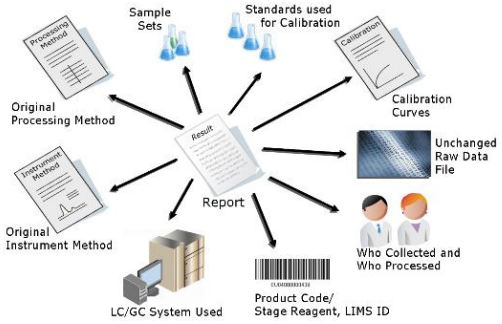
Example: DI and NIR Spectrophotometer



Example: DI and Networked Lab Data Acquisition System – HPLC/GC/LCMS

Data that must have integrity

1. Instrument control file
2. Run/Sequence File
3. Run Conditions
4. Acquisition Parameters
5. Integration Method
6. Chromatographic data
7. LC Calculations
8. Calculation Spreadsheets
9. Individual results and SST
10. Reportable Results



What must be part of ALCOA:

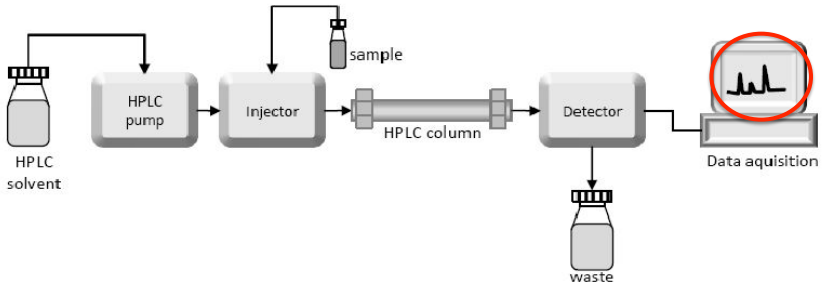
- Audit Trails
- for chromatographic run
- Meta data
- Processed data/results

Example: DI and Networked Lab Data Acquisition System – HPLC/GC/LCMS

1. Instrument control file: *records flow rate, temperature, wavelength etc….*
2. Run/Sequence File: *Sample ID, Order of injections, injection vol. etc…*
3. Run Conditions: *Metadata: sample ID, weights, ref. stds, dilutions,*
4. Acquisition Parameters: *specify parameters to record, sampling rate etc….*
5. Integration Method: *automatic or manual integration parameters ….*
6. Chromatographic data: *peak area, peak height, retention time etc….*
7. LC Calculations: *SST, calculations, meets accept criteria, etc….*
8. Calculation Spreadsheets: *export data to a spreadsheet to calculate etc….*
9. Individual Results and SST: *summary of individual results, run acceptance*
10. Reportable Results: *summary result matched to sample etc….*
11. Audit Trail: *date time stamp, analyst, changes etc…*

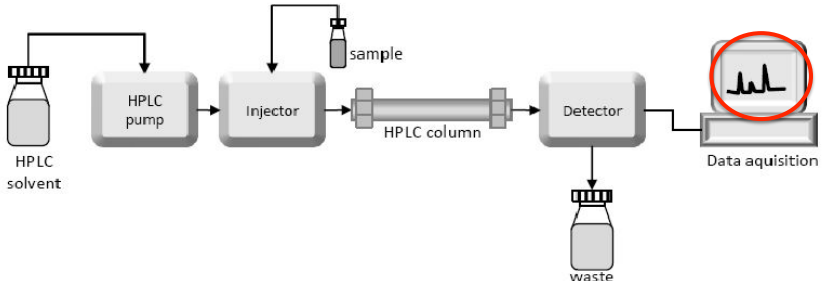
All the above raw data/metadata must be ALCOA to support a lab. result

Problem of Evaluation or "Trial" Injections



- No sample can be deleted, discarded or ignored - cannot selectively include or exclude data once its acquired;
- Cannot arbitrarily accept or delete the sample result, depending on whether it passes, or not;
- Running "trial" **test samples** is forbidden to "evaluate" the set up;
- Running trial, or evaluation **standards**, is OK – provided the evaluation step is documented in the written method and is a normal part of the method.
- The maximum # of evaluation trials must be documented in advance

Problem of Manual Integration



- Integration normally should be automatic, and not able to be changed by analyst;
- If subsequent manual integration is permitted there must be an SOP defining under what conditions;
- Manual integration should be accompanied by an OOS investigation.
- Manual integration can be used to alter a failing result to a passing result
- **Cannot alter integration parameters for one peak and not others**

2nd Person Checks of Lab. Data

- ***"The initials or signature of a 2nd person showing that the original records have been reviewed for accuracy, completeness and compliance with established standards"*** ***FDA*** (USP 211.194(a)
- The second (independent) person is critical to ensure data integrity and to confirm calculations are accurate.
- The 2nd person should review metadata and audit trail entries for modifications or deletion of data – to show the data is complete.
- Data entry into stability trial databases…is this double checked?

Laboratory Calculations and Spreadsheets

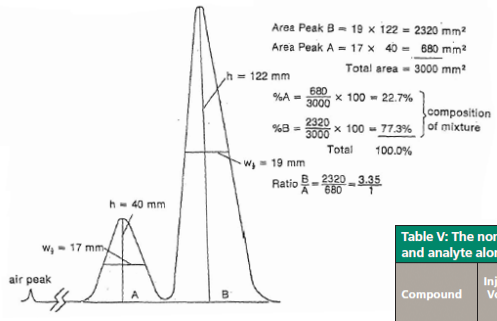


Table V: The nominal injection volumes, and exact masses and volumes of IS and analyte along with the resulting response factors

Compound	Injection Volume (µL)	Avg. Mass (g)		Avg. IS Volume (solution) (mL)	Avg. Peak Areas		Response Factor
		IS Solid	IS solution		Analyte	IS	
Indoxacarb*	4.0	0.055	0.008	15.5	6000	4000	1.50
Indoxacarb*	4.0	0.050	0.010	15.3	3687	3627	1.02
Indoxacarb†	5.0	0.100	0.012	7.9	7100	1500	4.73
Famoxadone	10.0	0.125	0.040	7.9	4100	4300	0.95
Diuron	2.0	0.100	0.100	7.8	4230	1830	2.31

*Pure active enantiomer
†75:25 racemic mixture of active and inactive enantiomers

Laboratory DI and Spreadsheets
(PICs/ TGA 2016)

- The laboratory uses a number of spread-sheets to calculate analysis results.
- The QC Laboratory Manager has access to protected cells containing formulations and calculation output.
- There was no SOP in place to ensure data changes were recorded and traced.

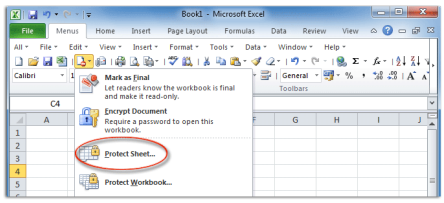
GxP Spreadsheets
(Lab and Production)

- Widespread use but sometimes hidden from view;
- Generally find 3 uses with increasing level of DI risk;
  - A. **Word Processing**
  - B. **History Record/Log/Register**
  - C. **Data Manipulation via Macros**
- Need an inventory of all spreadsheets sheets classified by risk;
- Need to lock up user access to A, B & C;
- **Need to validate C;**
- Need to place the spread-sheet under change control and verify periodically;

Spreadsheet Protection and Changes Tracking

A	B	C	D	E	F	G	H	I	J	
tion mber	Date	Time	Who	Change	Sheet	Range	New Value	Old Value	Action Type	Losir Actio
1	23/11/16	6:47 PM	Steve Williams	Cell Change	Summary Sheet	H11	Not complies	Complies		
e history ends with the changes saved on 23/11/2016 at 6:47 PM.										

- XL spreadsheets have some functions that support Audit Trails – **Turn on Track Changes** and activate "History" file – the file cannot be altered once turned on.
- XL has file, sheet and cell protection which locks cells from changes without knowledge of the password.

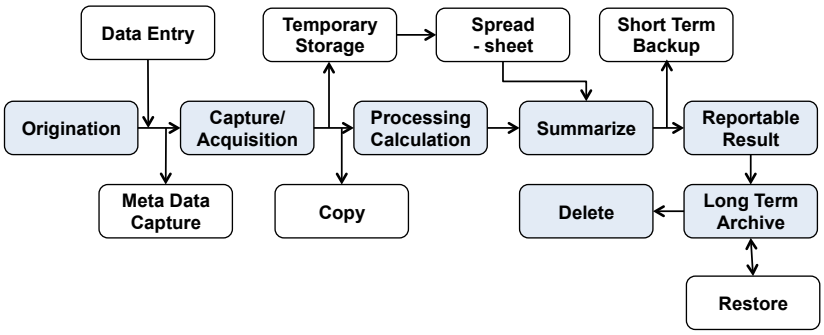


Some Recommended Laboratory SOPs

- Laboratory Notebooks - control and recordkeeping requirements
- Validation, Verification and Use of GxP Spreadsheets
- Control and Change Management for Laboratory computerised systems eg. Data Acquisition Systems
- Rules for Rounding and Significant Figures
- OOS Investigations
- Chromatographic Integration and Reporting
- Requirements for Statistical Analysis and Reporting
- Data Integrity Requirements/Policy
- Backup, Security and Restore of Electronic Records
- Changing Data Entries or Results once they are approved

Assessing Risk of DI Vulnerability

1. Map data lifecycle in a flowchart



2. Analyse each step for DI vulnerability or risk

Mapping Process Vulnerabilities

Process Step	Initiate →	Acquire →	Process →	Calculate →	Report →	Archive
Where from/to ? Storage media						
Metadata / Audit Trail						
Human Access Manipulation						
Calculations Summaries						
Security Level Static/Dynamic						
Other Information						

Understanding Vulnerability- Checksheet

Vulnerability Assessment Checklist				
	Describe the Data Flow Process Step(s). 	Risk		
		High	Medium	Low
1	Does the data originate from a validated source / instrument / equipment ?	☐	☐	☐
2	Is the data stored permanently (becomes static) ?	☐	☐	☐
3	Is the original data and meta data etc. captured in static form at these steps ?			
4	Is there metadata / audit trail associated with the data ? How extensive ?	☐	☐	☐
5	Is the metadata / audit trail captured and protected from change ?	☐	☐	☐
6	Is the data captured by a human ? or machine/instrument ?	☐	☐	☐
7	How is the data passed between sequential process steps ?	☐	☐	☐
8	Is the data available for alteration/change during transfer ?	☐	☐	☐
9	In which process step/system is the data processed ? is this step validated ?	☐	☐	☐
10	Are the established password access controls to protect the data ?	☐	☐	☐
11	Is the data transformed or migrated at this step ?	☐	☐	☐
12	Is there analogue to digital conversion of the data at these steps ?	☐	☐	☐
13	Is there a human data entry step ? Is there a double check in accuracy of entry ?	☐	☐	☐
14	Can the data be modified at this step ? if so is it audit trailed ? Password access ?	☐	☐	☐
15	Is there data editing required ?	☐	☐	☐
16	Is the data calculation / summary step manual or automated ? double checked ?	☐	☐	☐
17	Can the data be electronically copied and exported ? Can it be deleted ?	☐	☐	☐
18	Other considerations ?	☐	☐	☐

Flash Quiz

	Regulatory / GMP Expectation for Risk Management	Your Selection
1	Which of these statements is true (there may be more than one) (a) Data Integrity (DI) has been an industry issue for over 30 years (b) DI issues are limited to India and China industry (c) DI regulations apply to GMP and GDP only (d) Application integrity reviews are part of FDA PAI inspections	
2	Which one of these statements is true: (a) Data Integrity issues are the concern of FDA inspectors, not WHO or PIC inspectors. (b) Data integrity issues are mostly confined to the QC laboratory. (c) Data integrity issues are of key interest to all regulatory inspections. (d) Data integrity is mostly a concern in clinical trial data not in manufacturing	
3	Use of eRecords and GMP software have made DI issues reduce	TRUE/FALSE
4	What does the term ALCOA stand for ?	

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Validation Strategies for Pharmacopoeial Test Methods

Module Topics

- Regulatory Positions
- Decisions on Validation
- Verification Requirements

Some Useful Reference Documents

- USP General Information-<1225>Verification of compendial procedures.
- USP General Information-<1226>Verification of compendial procedures
- 21 CFR 211.194 (a), and 194(a)(2)
- ICH Guidelines series ICH 1 through to ICH 6(b)
- FDA Guidance-Analytical Procedures and Methods Validation for Drugs and Biologics 2015
- WHO-(DRAFT) Guidelines on Validation – APPENDIX 4, Analytical Method Validation (June 2016)
- British Pharmacopoeia, European Pharmacopoeia.
- WHO-International Pharmacopoeia

Legal Standing of Monograph Tests

- *Monographs are official standards in the jurisdictions they are written for.*
- *For example in the EU:*
  - *"The pharmaceutical legislation in the European Union makes monographs obligatory standards (2001/83/EC, 2001/81/EC)"*
- *For example in the USA:*
  - *"assays and specifications in monographs of the United States Pharmacopeia and the National Formulary constitute legal standards."*
- So if you are supplying to a "WHO" target country ensure you know which Pharmacopoeia they are using- usually some historical ties to colonial era (BP, USP, Ph Eur).
- Some "WHO" target countries may simply adopt the "Pharmacopoeias or equivalent" stance.

What the Regulators Say- FDA

- USP<1226>39 Verification of Compendial Procedures

"Verification consists of assessing selected analytical performance characteristics, such as those that are described in chapter <1225>, to generate appropriate, relevant data rather than repeating the validation process."

- Under 21 CFR 211.194(a)(2)

" the suitability of all testing methods used shall be verified under actual conditions of use."

- Does not cover Microbiological testing as they have their own chapters.

What the Regulators Say- FDA

- Assumptions being made <1226> (Caution!)

"*Users should have the appropriate experience, knowledge, and training to understand and be able to perform the compendial procedures as written. Verification should be conducted by the user such that the results will provide confidence that the compendial procedure will perform suitably as intended."*

- Analysts can rarely pick up a compendia and "just test RFT" particularly biological assays.
- <1226> makes it clear that one needs to liaise with the USP on resolving issues BEFORE you start to use an in-house method (which may become a new USP method).
- Assume this is the similar situation within the UK/EU

What the Regulators Say- FDA

- Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry (2016).
  - Does not specifically cover biological assays, immunogenicity or animal challenge studies (This means you need to demonstrate suitability of the specific elements keeping the overall intent in mind; it does not mean you do not need to do it).
  - Submissions of analytical methods as part of NDA, ANDA, BLA should contain all the required information for the FDA to then recognise the method as "approved".
  - "demonstrate the manufactured product meets prescribed standards of identity, quality, safety, purity, and potency."
  - If you use a compendial method to test another or new product then you must validate its use in the new product matrix.

What the Regulators Say- EU

- *ICH Q2 "This document presents …….the characteristics for consideration during the validation of the analytical procedures included …… registration applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world."*
- *Ph Eur "The procedures for the tests and assays published in the individual monographs have been validated according to current practice at the time of their elaboration for the purpose for which they are intended."*
- *Ph. Eur. "tests are reference methods, essential in cases of dispute Compliance is required, but alternative methods may be used as long as they lead to the same pass/fail result. It is the responsibility of the user to demonstrate their suitability. Approval of the competent authority is necessary in many cases."*

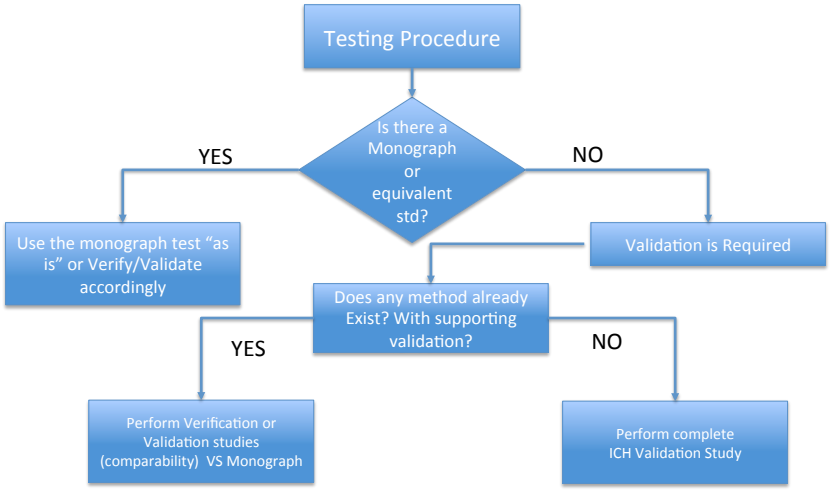
What the Regulators Say- WHO

- Draft Guidelines for Validation – App 4 Analytical Method Validation (June 2016)
- Pharmacopoeial methods are acceptable as well as "National regulatory agency" approved methods.
  - This is in line with FDA, EMEA, TGA as the term "approved" covers the caveats previously mentioned ie supported by appropriate validation.
- Pharmacopoeial methods must still have supporting evidence of suitability under conditions of use.
- Non-Pharmacopoeial methods should be appropriately validated.
- Expectation that if a non-pharmacopoeial method is used that there is some cross validation/comparison with the pharmacopoeial method along the lines of a method transfer i.e. provide identical results, ANOVA testing etc.

What must comply?

- All substances for Pharmaceutical use:
  - Starting materials
  - Ingredients
  - Excipients
  - Solvents
  - Buffer ingredients
  - Primary Packaging (Vials, Plastics)
- For synthetic API, Biotechnology (Cell culture) if there are Pharma grade materials you are expected to use them.
- Upstream and chemical components used in API, you can use "technical grade" if there is no Pharma grade available.
- Note: *you cannot simply test a technical grade into Pharma grade as it has not been manufactured under GMP conditions.*
  - If you audited a BP grade supplier of Glucose versus technical grade and they are ISO 9001 certified (often the case), auditors may be lenient; however this is more likely for non-compendial materials then well known materials which have a clear compendial history.

Decision Tree



Compendial Assay Verification

Verification?

- Choose according to complexity of assay.
- Training?
- Equipment?
- Experience?
- Sample matrix/different excipients?
- Risk Assessment?
- Pick carefully…..

Baseline Criteria

Parameter	ICH	USP	WHO
Specificity	☑	☑	☑
Accuracy	☑	☑	☑
Precision: Repeatability	☑	☑	☑
Precision: Intermediate precision	☑	☑	☑
Precision: Reproducibility	☑	☑	☑
Detection Limit	☑	☑	☑
Quantitation Limit	☑	☑	☑
Linearity	☑	☑	☑
Range	☑	☑	☑
Robustness	☑	☑	☑

Compendial Assay Verification

Verification?

- You will try the compendial method initially to evaluate its performance with your product.
- From that point focus on what are seen as problem areas- Experience/Training focus on Precision/ Robustness.
- Sample matrix effects- focus on Specificity, detection, quantitation, spiking studies.

Baseline Criteria

Parameter	ICH	USP	WHO
Specificity	☑	☑	☑
Accuracy	☑	☑	☑
Precision: Repeatability	☑	☑	☑
Precision: Intermediate precision	☑	☑	☑
Precision: Reproducibility	☑	☑	☑
Detection Limit	☑	☑	☑
Quantitation Limit	☑	☑	☑
Linearity	☑	☑	☑
Range	☑	☑	☑
Robustness	☑	☑	☑

Comparability

- An approach similar to inter-laboratory transfer may be used- we are comparing two methods meant to produce identical results.
- Performance Criteria:
  - Precision- RSD NMT 2.0%
  - Linearity-Using regression analysis, correlation coefficient r^2 NLT 0.985
  - Range-each percentage RSD, calculated from triplicate injections of solutions at 75% to 125% of expected concentration; RSD should be NMT 2.0%.
  - Ruggedness- The results from Analyst 1 shall be not statistically different from Analyst 2.Day to day, instrument to instrument, sample hold times etc. etc.
- You should know your assay, so challenge it.

	Compendial Methods	Your Selection
1	Which one of the following statement is true: a) If performing a compendial test exactly as written on a raw material it does not require validation. b) If an in-house assay has been developed that uses more modern methods it can be substituted without and cross validation. c) If an in house test has been substituted for a compendial test, the test object does not need to pass the compendial method as well. d) If a HPLC test has been introduced in a compendia for related substances for a compendial antibiotic, it means that you no longer need to conduct the bioassay but just use HPLC.	
2	Choose the two True statements from the following: a) If your company is in the developing world and has not historically followed any particular compendia, you can use the WHO international compendia for product to be used domestically. b) If your company is in a PIC's country you can use either the BP or Ph Eur. c) If you use the USP to test your product you can market it anywhere. d) If you are in a developing country and have agreed with the national regulatory authority you can adopt a compendial method from any of the recognised compendia.	
3	Choose the False statements from the following: a) If you are following a compendial method directly from the compendia you do not need to write down your steps, weighing's, dilutions and calculations in a lab book. b) When conducting a compendial assay you do not need to validate the spreadsheet used to calculate the results. c) When conducting a compendial assay it is ok to modify the sample preparation volumetric dilutions so long as the concentrations are equivalent. d) Bioassay method updates in compendial tests should have some verification comparison testing done before adopting for general use.	

FLASH QUIZ

79

CBE
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Management And Investigation Of Out Of Specification (OOS) Events

Module Topics

- Historical Perspective
- Current Regulatory Thinking
- OOS Investigation Methodologies
- OOS Investigation of Biological Methods

Some Useful Reference Documents

- Clin. Res and Reg Affairs (10) 3, 137-157 (1993) Abstract "The ever changing standards of CGMP- USA Vs Barr Labs Inc"
- WHO Technical Report Series, No. 957, 2010, good practices for pharmaceutical quality control laboratories
- FDA Guidance For Industry-Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production (CDER 2006)
- MHRA Presentation- Out of Specification Investigations 2013 (Gov.Uk)
- PICS Aide Memoire-Inspection of Quality Control Laboratories, 9.2 Failures -OOS

Summary of Barr Decision Findings

Findings

- Testing into compliance
- Averaging bad with good to pass
- FDA any one unit fail, batch fail
- Informal and formal investigations
- Testing and retesting
- Sampling and resampling
- Averaging
- Inappropriate outlier testing
- Product “failure”
- Sampling and resampling

Judgement

- Not permitted to average OOS results with in-Specification results to get a Passing Result.
- Not permitted to conduct multiple retests with no predetermined limit.
- Outlier tests cannot be used to reject results without due cause in chemical testing (silent on Biologicals)
- Companies must have an OOS policy and procedure.

FDA Guidance 2006

- Applicability:
  - Chemistry-based testing of drugs regulated by CDER including Biologicals. Does not cover Biological in Vivo assays.
  - Tests that are performed on API, excipients and other starting materials, in-process testing, and finished drug products.
  - CMO purchased products but tested in house.
  - Contract laboratories performing testing on any of the above on companies behalf.
  - Testing results obtained as part of stability trials, final process PV validation studies, inprocess monitoring, critical utilities (Water, Air, Gases).

What is an OOS?

- In pharmaceuticals, the term OOS applies to all test results that fall outside the specified acceptance criteria defined in:
  - The Pharmacopoeial Specifications
  - Drug master files
  - Registration dossiers/marketing authorisations
  - Finished dose specifications
  - Inprocess specifications (Not routine adjustments)
  - Stability trial specifications
  - Final process validation protocols for saleable product.
  - Water, Air, Environmental monitoring
  - Active pharmaceutical ingredients, excipients
  - ……

What is an OOS?

Other terms that are appearing are:

- Out of trend (OOT) -Is generally a time dependant stability result (ICH Q1b) that does not follow the expected trend;
  - In comparison with other stability batches or with previous results collected during a stability study.
- However the trends of starting materials and in-process samples may also yield out of trend data. The result is not necessarily OOS but does not look like a typical data point and may require investigation (vendor assurance for raw materials, rotational testing etc..)
- Out of expectation (OOE) -Results that are still within specification but are unexpected, questionable, irregular, deviant or abnormal. Strange results during OQ/PQ, a sudden step change, still within specification (sometimes post calibration, or new ref std introduction)
- We will **focus on OOS**.

Flash Quiz

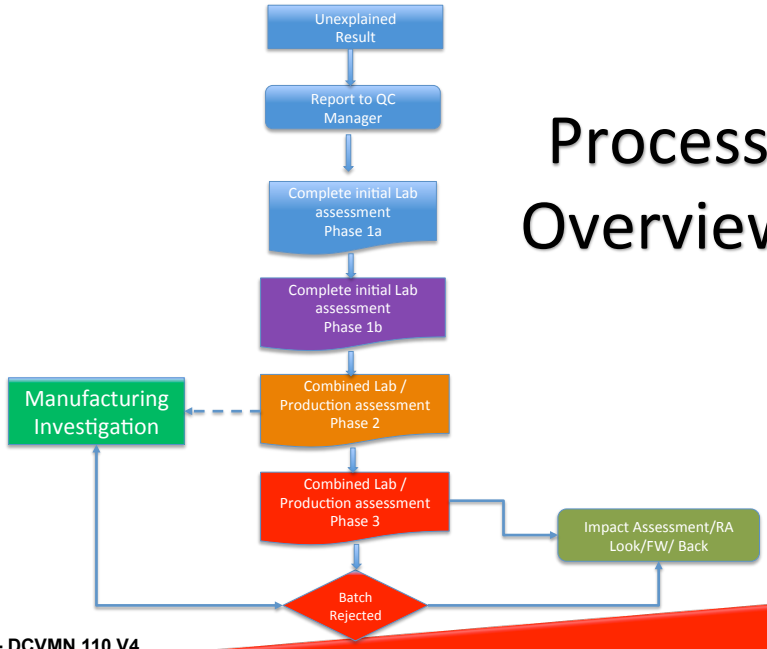
	OOS Investigations	Your Selection
1	Which one of the following statement is most correct: a) The approach for OOS is the same for HPLC as it is for Bioassays b) Bioassay monographs often allow for the application of the outliers test to remove data points c) The outliers test, if used for bioassays should only be applied to the standard set as well as the test data set d) The Monographs for bioassays specifically preclude the use of outliers tests as they introduce bias into the method	
2	Which one of the following is a potential OOS? a) Environmental monitoring had a viable count spike in the water just below the action limit? b) A new source of API has been qualified and a new EP standard obtained. The API does not have any of the usual related substances or any other for that matter but the EP std still does. c) An analyst is conducting a LAL Endotoxin assay and takes over from morning shift who was supposed to de-pyrogenate the glass vials 250C for 30 mins. Not sure if that step was done (as they are purchased depyrogenated but pack open already), test is conducted and fails Endotoxin test. d) In process pH of bulk solution fails in QC Lab but passes using production pH meter. Production proceeded to fill without waiting for result. QC pH calibration check satisfactory.	

Laboratory Investigation

- Using the FDA/MHRA model there are 4 stages to the investigation:

Stage	Activities/Purpose
Stage 1a	To establish if there have been any clear assignable cause such as power failure, sample spilled etc.
Stage 1b	OOS identified but source is not clearly identified, laboratory based investigation needed.
Stage 2	Investigation now includes manufacturing, no clear source of lab error; Manufacturing investigation conclusion required before any resamples taken. Plan needed and hypothesis.
Stage 3	Full report required from Lab and Production even when batch rejected.

Process Overview



Out of Specification Conditions (Simple Key Decisions)

- Was it a laboratory error ?
- Does a retest confirm the original result ?
- Does a resample confirm the original result ?
- Is the test system reliable ?
- Is there a reliable history - product - test ?
- Is the sample representative of the batch ?
- Is the batch homogeneous ?
- Do the manufacturing records indicate error ?
- **There is a stepwise process for conducting these steps!**

Basics of investigation

Not so common sense!

NEVER knowingly produce and OOS

- If the run acceptance criteria are not met, i.e. system suitability, drift (large runs), standard curves r^2 etc..
- Continue with tests that you know have had an error or mistake performed and hope it will pass (missed a "time critical" step in the test, incorrect dilution, or unsure…).
- Not all analysts are the same, some are adept at very painstaking and sensitive precise testing- others are not, be discriminating as required.
- Staff should not be working when judgement is impared.

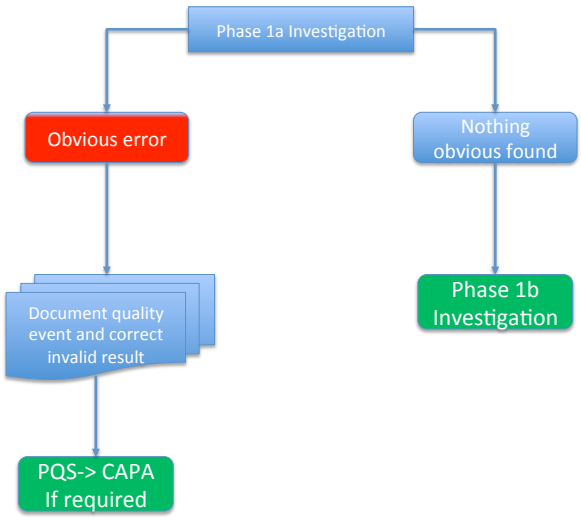
Basics of investigation

- The investigation must be according to written instructions and:
  - Follow predetermined and structured plan.
  - As an output produce a well documented and summarised report.
  - The Report shall be sufficient to support product release AND any subsequent CAPA/Change controls.
- The investigation should look forward and look back in that:
  - Trends should be reviewed for OOS from common instruments/ methods.
  - Trends from common products and analysts.
  - Other potential trends or changes (night shift?).
  - Impact on inventory, starting materials, inprocess, WIP, finished goods, recall?

Phase 1a

Phase 1a Obvious Laboratory Error

- Good GLP requires that all samples and test solutions are retained until all results and calculations have been checked and approved. Even if there is no OOS.
- The standard sample size should be sufficient to cover the event of an OOS WITHOUT RESAMPLING (Check!); as well as the retained sample.
- If possible, the initial investigation should be done before test preparations (including the composite or the homogenous source of the aliquot tested) are discarded.
  - Basic calculation error found on checking, correcting according to GDP.
  - Power failure and analysis interrupted- (power outage SOP)
  - Other equipment failure- obvious leak in HPLC, dropped sample flask, used wrong pipette, incubator door was not closed etc.
  - Testing error- sampling time incorrect (kinetic study, dissolutions, LAL), contaminated, simple mistake (missed step in method), clumsiness (dissolution sampling).
  - Instrument setup incorrect- incorrect column, method use, pipette out of calibration.
- If none of above (see checklist) move to 1b.



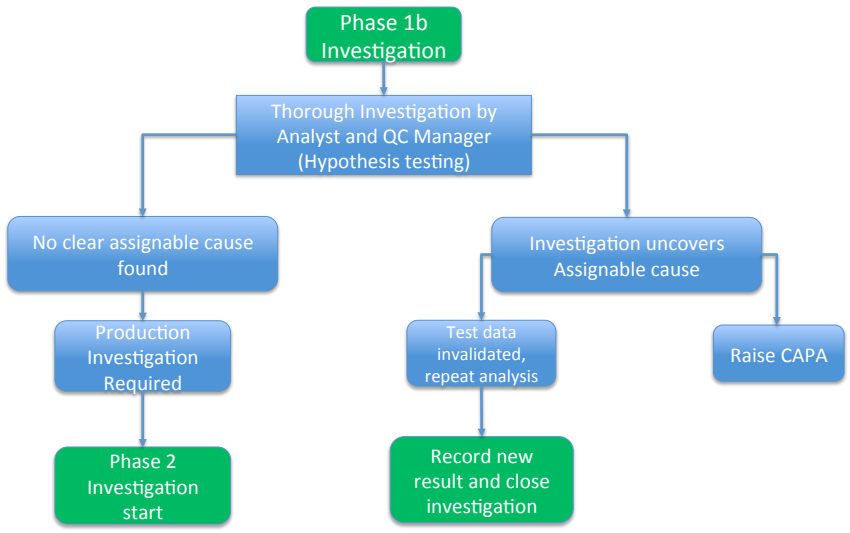
Phase 1b

Phase 1b Indeterminate Laboratory Error

An OOS Investigation Report should be raised and a formal investigation commenced. The QA Manager should also be immediately informed. This should be done before any re-testing or re-sampling is carried out.

The following steps should be taken as part of the supervisor's formal assessment:

- Discuss the test method with the analyst; confirm analyst knowledge of and performance of the correct procedure. Review their training records.
- Examine the integrity/preservation of the sample
- Examine the raw data obtained in the analysis, including chromatograms and spectra, and identify anomalous or suspect information.
- Confirm the performance of the instruments.
- Determine that appropriate reference standards, solvents, reagents, and other solutions were used and that they meet quality control requirements.
- Evaluate the performance of the testing method to ensure that it is performing according to the standard expected based on method validation data.
- Document and preserve evidence of this assessment.
- Any other relevant evidence based on the above steps, enabling the supervisor to allocate a category to the OOS in order to proceed to further investigation.



1b Methodology

1. Initial 1a checks completed and analysts interviewed by QC Manager; should be restricted to data, calculations, instrumentation only initially.
2. QA and Production is informed.
3. For microbiological OOS ensure all items related to the test failure are retained:
   a) Relevant environmental plates samples (No implicated test environmental plates should be destroyed until the investigation has been completed).
   b) Dilutions, ampoules/vials of product, temperature data, autopipettes, reagents – growth media.
4. Once QC Manager and analyst have reviewed all available information and developed AND documented a hypothesis investigational testing only can commence to test the hypothesis but at this stage can only include testing of original sample.

Phase 1b Checklist-Chem

CHECKLIST OF REVIEW OF CHEMISTRY RESULTS (*TICK APPROPRIATE BOX*)

Was the correct laboratory test method followed explicitly?	Yes ☐ No ☐ N/A ☐
Were the correct dilutions made?	Yes ☐ No ☐ N/A ☐
Was sampling done correctly?	Yes ☐ No ☐ N/A ☐
Was instrumentation used in testing suitably calibrated at the time of testing?	Yes ☐ No ☐ N/A ☐
Is the Instrument in correct working order?	Yes ☐ No ☐ N/A ☐
Was the correct reference standard used?	Yes ☐ No ☐ N/A ☐
Was the reference standard suitably qualified?	Yes ☐ No ☐ N/A ☐
Did the test method perform as expected? If HPLC analysis was involved did the system suitability test meet acceptance criteria?	Yes ☐ No ☐ N/A ☐
Are the calculations correct?	Yes ☐ No ☐ N/A ☐
Review Raw Data and any charts.	Yes ☐ No ☐ N/A ☐
For HPLC analysis is the type of integration consistent between samples & standards	Yes ☐ No ☐ N/A ☐
Were all reagent solutions used within their expiry dates	Yes ☐ No ☐ N/A ☐
For volumetric analysis how long ago was the standard solution standardised?	Yes ☐ No ☐ N/A ☐
For multiple analytes assayed by HPLC has the test method been suitably validated?	Yes ☐ No ☐ N/A ☐
Are there any errors of transcription?	Yes ☐ No ☐ N/A ☐
Was the sample stored correctly	Yes ☐ No ☐ N/A ☐
Have the correct limits/specifications been applied?	Yes ☐ No ☐ N/A ☐
Are there any other sources of analysis error?	Yes ☐ No ☐ N/A ☐
Is there any possibility of method imprecision?	Yes ☐ No ☐ N/A ☐

Phase 1b Checklist-Micro

CHECKLIST OF REVIEW OF MICROBIAL RESULTS(*TICK APPROPRIATE BOX)*

Was the correct laboratory test method followed explicitly?	Yes ◻ No ◻ N/A ◻
Were the correct dilutions made?	Yes ◻ No ◻ N/A ◻
Was sampling done correctly?	Yes ◻ No ◻ N/A ◻
Was the sample stored correctly?	Yes ◻ No ◻ N/A ◻
Has any incubator / autoclave malfunctioned?	Yes ◻ No ◻ N/A ◻
Were the correct controls used?	Yes ◻ No ◻ N/A ◻
Were controls suitably qualified?	Yes ◻ No ◻ N/A ◻
Are the calculations correct?	Yes ◻ No ◻ N/A ◻
Was media used suitably qualified when first prepared?	Yes ◻ No ◻ N/A ◻
Were all media / reagent solutions used within their expiry dates?	Yes ◻ No ◻ N/A ◻
Has the test method been suitably validated?	Yes ◻ No ◻ N/A ◻
Are there any errors of transcription?	Yes ◻ No ◻ N/A ◻
Are there any other sources of testing error?	Yes ◻ No ◻ N/A ◻
Have the correct limits/specifications been applied?	Yes ◻ No ◻ N/A ◻
If a heating block was used has it been calibrated (for temperature)?	Yes ◻ No ◻ N/A ◻
If the failure involves biological indicators were the indicators qualified before use?	Yes ◻ No ◻ N/A ◻

1b Outcomes

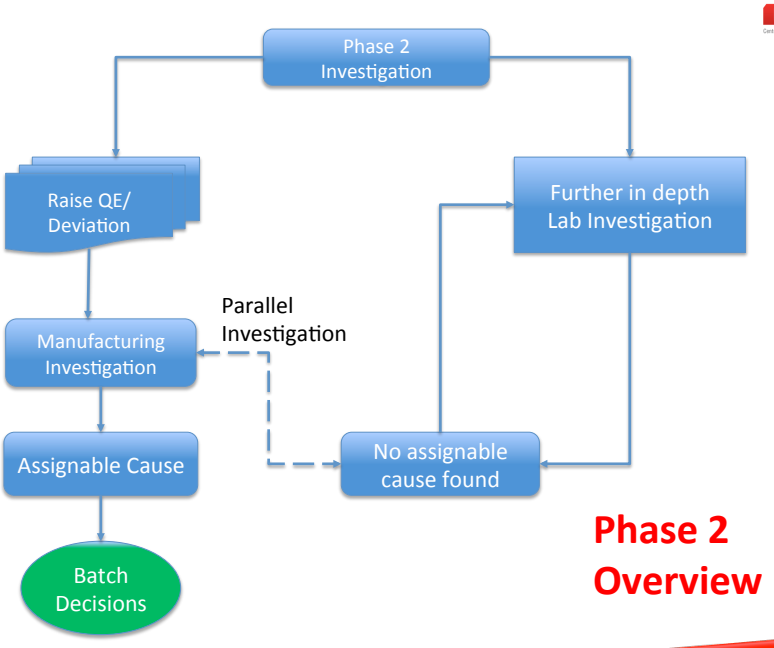
- **Assignable Cause** – An identified reason for obtaining an OOS or aberrant/ anomalous result. Hypothesis testing shows that for example a sample filtration or sonication was not complete or correct (same sample).
- **Lack of assay precision*** (If a test method is validated this category will not apply as the Method Precision will be known and acceptable to the application. If the precision of the method is not known (i.e. method not validated) then individual results may fall outside the specifications by chance alone due to inherent variation within the assay.
- **No Assignable Cause** – When no reason could be identified, move to step 2.
- **Invalidated test** – A test is considered invalid when the investigation has determined the assignable cause.
- **Reportable result** – Is the final analytical result. This result is appropriately defined in the written approved test method and derived from one full execution of that method, starting from the original sample. Original result stands.
- **Warning Level or Trend excursions** – If two or more consecutive samples exceed warning (alert), or if an increasing level of counts, or same organisms identified, over a short period was identified consideration should be given to treat the results as action level excursions.

If none of above (see checklist) move to 2.

* May be valid in clinical phase 1-2

Flash Quiz

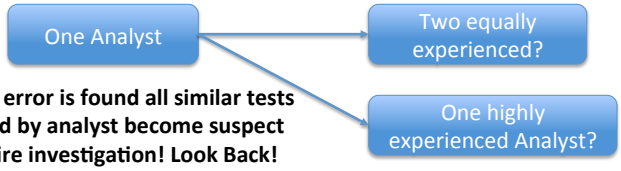
	OOS Investigations	Your Selection
1	Which of the following statements is acceptable true/false: a) The QC lab sample was submitted to the lab during night shift and instead of being refrigerated (as per method/specification) it was left in the sample transfer area for 6 hours at room temp. QC manager raised a deviation and ordered a resample of the bulk solution. b) The analyst was in a hurry and instead of making a new std curve used the previous one (but not freshly prepared as specified). The test failed and analyst submitted an OOS. c) The analyst sonicated the inprocess sample and filtered (filter paper) for UV assay but the absorbance was too high, knowing it was interference he drew some sample through a HPLC filter instead and the result came into normal range production, proceeded to next step. Final sample tested by another analyst by usual method and the batch failed, OOS raised. d) After reporting a catastrophic failing assay (<75%LC) and discounting all obvious sources of lab error the QC manager decided to inform the QA manager that they have progressed to a phase 1b investigation which revealed a second source of API was being used. Knowing a production campaign was underway QA informed production before the next batch is started as an intervention. Soon after QC noted that an incorrect potency was entered into the MRP system so genuine OOS likely.	



Phase 2 Overview

Phase 2 Methodology

- Phase 2 investigation starts when no clear laboratory error has been found. No more sampling and testing is allowed until manufacturing have conducted their investigation; and then only to an approved hypothesis testing protocol.
- At this stage the hypothesis may include using alternative analysts (original sample) i.e.:



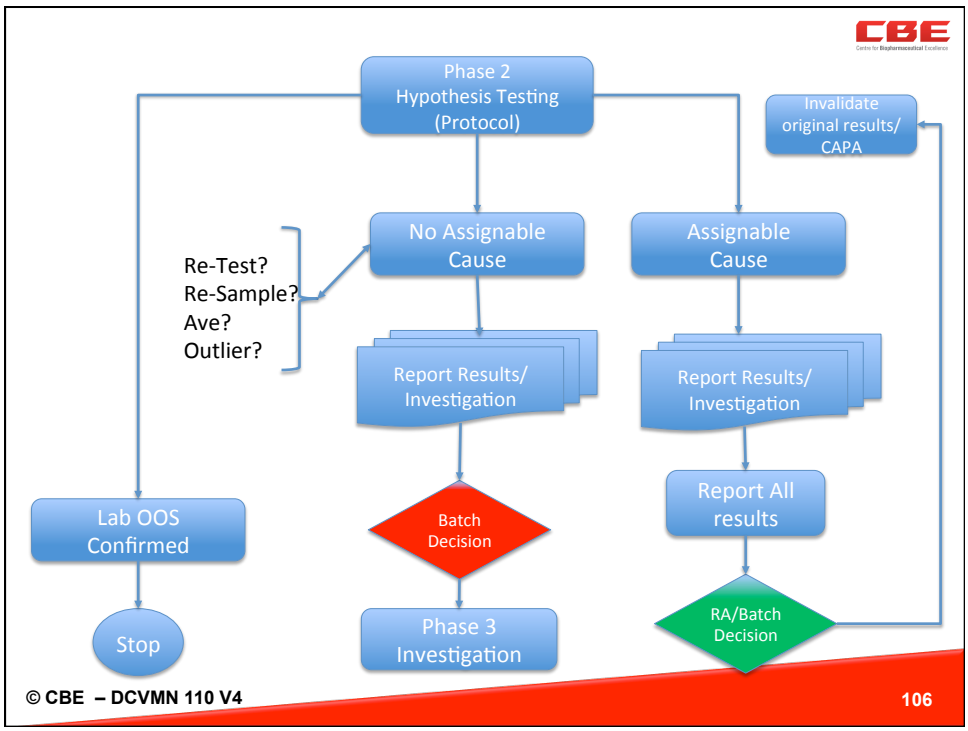
If analyst error is found all similar tests conducted by analyst become suspect and require investigation! Look Back! Not just OOS results!

Phase 2 Hypothesis Testing

- Hypothesis can be developed during phase 1 and finalised in phase 2 should be documented and include:
  - What root cause, will the hypothesis reveal?
  - What samples to be tested and how many?
  - The exact testing to be conducted and in what order?
  - How the data and results will be treated and finalised?
- Re-testing requires using part of the original sample supplied to the lab or if insufficient remaining re-sample from the identical container/bulk in production (without further mixing/blending!).
- Re-sampling allowed only if insufficient original sample or if a know sampling/homogeneity issue is suspected (production investigation may show this depending on who takes samples).
- Hypothesis testing (investigative testing) can include in the written plan:
  - Re-filtration of sample (original or freshly prepared from original submitted sample).
  - Re-sonication of same sample (original or freshly prepared from original submitted sample).
  - Checks of equipment malfunctions (calibrations- it may still be within calibration but malfunctioned recently).
  - Checks using stability sample/ Known test batch samples/stds (if such a system is documented).
  - Running spiked sample/placebos/standards to check validation parameters.
  - Can be many things but must be based in science and empirical evidence.
- If hypothesis testing evidence supports a conclusion then this would be regarded as the most *probable cause* of laboratory error i.e. secondary standard was found to have absorbed moisture after standard check versus new vial and KF.
- The original result cannot be excluded unless a clear laboratory or sampling error is revealed in stage 2.

Phase 2 Sample Details

- The typical OOS sampling protocol should be documented in the OOS SOP.
- Sampling considerations include:
  - Testing of original sample (prepared test solution) not a different sample.
  - If original test solution is unavailable (insufficient, expired) then a new test solution made from original supplied lab sample can be prepared.
  - If there is not enough original sample QA must approve equivalent resample from exact location if possible. Exact details of this must be recorded (bulk solution may have been transferred to another tank etc.).
  - The number of retests must be scientifically justified and approved by QA in the test plan before commencement.
  - Since the Barr decision the industry practice and various papers suggest a minimum of 5, 7 or 9 retests are required (duplicate, triplicate?). The original results cannot be discarded but must be included unless logically excluded during phase 1a/b.
  - It is suggested that an alternative and or more experienced analyst is used, baring in mind that if the retests pass then the investigation needs to then demonstrate what the initial analyst did incorrectly and review other analysis conducted by that person.



Phase 2 Averaging of Results

- So what to do with the 5,7,9 new results?
- Averaging of results is normal for example:
  - HPLC results
  - Microbiological assays
  - LAL Endotoxin testing and other "well based" testing
- This is valid unless there is clear variation between replicates of the same sample- in which case this should also be investigated as part of the hypothesis.
- Averaging cannot be used where the test is specifically for detecting homogeneity issues- content uniformity, inprocess blend testing.
- It is often preferable for investigation results to report them all individually including original and having them individually approved by QA prior to averaging. It is forbidden to average an OOS result into specification by simply overwhelming the original value.
- Sometimes statistical treatment of the results is required particularly in Micro and Biological assays.
- Use of the "Students t test" and 95% confidence limit testing may be used to show the variability of averaging.
- Outlier testing may be used in Micro and Biological assays, less justifiably with chemical assays (see later).

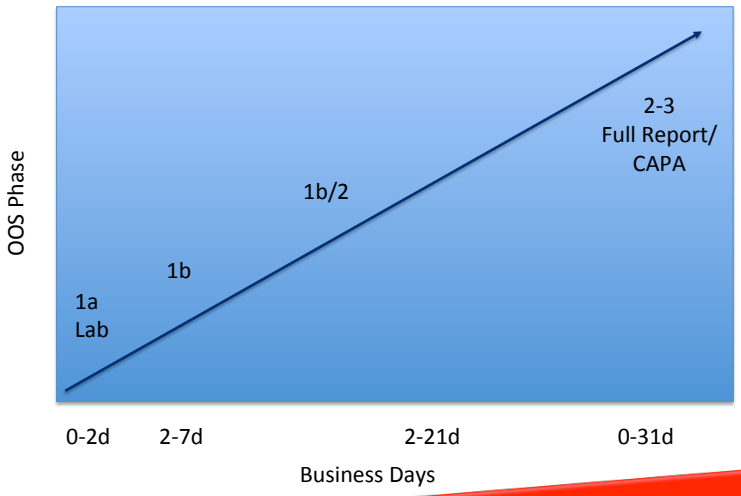
Outlier Testing

- An proven outlier result can come the testing or from the sample itself- one cannot presume it's the method.
- Statistical outlier testing can be justified in inherently variable testing environments such as Microbiology and Biological assays and its performance and requirements are detailed in the Pharmacopoeia USP, BP, Ph Eur.
- However, for validated *secondary* chemical methods due to the inherently low variance it cannot be used to reject chemical data. It cannot be used at all for *primary* (molar/ stoichiometric) methods at all.
- **Outlier Test will not identify the cause of an extreme observation and, therefore, should not be used to invalidate the suspect result.**

Flash Quiz

	OOS Investigations	Your Selection
1	Which one of the following statement is most correct: a) The approach for OOS is the same for HPLC as it is for Bioassays b) Bioassay monographs often allow for the application of the outliers test to remove data points c) The outliers test, if used for bioassays should only be applied to the standard set as well as the test data set d) The Monographs for bioassays specifically preclude the use of outliers tests as they introduce bias into the method	
2	Choose one True statement from the following: a) If a biological assay fails by junior analyst (A) due to not meeting acceptance criteria but passes by senior analyst (B) the reason must be analyst error or training. b) When conducting repeat testing you just need to overwhelm the OOS result i.e. conduct 5 repeats and average all results including the original OOS. c) If a biological assay fails, but also fails the test acceptance criteria i.e. %CV <20% for Endotoxin test fails, then it is not an OOS but an invalid test. d) Using the Dixons outlier test is the 1st step in investigating a biological OO	
3	Choose the one True statement from the following: a) Biological assays (bioassays) are less robust than equivalent chromatographic methods. b) Bioassays using animal models (in-vivo) are generally more reliable than in-vitro methods. c) Bioassays should always be repeated 3 times to improve accuracy. d) Bioassays should only be repeated if the initial assay is out of specification.	

Timeline Targets



Phase 3

- Phase 3 should review the whole investigation package and draw upon subject matter experts and R & D etc..
- Depending on outcome production may need to be suspended.
- Once the batch has been rejected there is no boundary as to what testing and experimentation can be done.
  - The batch cannot be resurrected as a result of further testing*.
- The impact of the OOS should be extended to a thorough investigation of potential impacts on other lots, in-process, product on the market, stability.

* Rework? 100% inspection for particulates?

Phase 3- Conclusions

- If no Lab errors are identified and the OOS stands, all results should be reported (including in C of A). And all data used in final decision.
- If investigation finds that there is an inherant sampling error and new method needed (use of riffle box, use of unit dose sizes), then the new method can be approved via change control by QA. Again look back?
- An initial OOS does not mean the batch fails and should be rejected- QA must evaluate all the findings to reach a decision, release or reject and fully document.
- In those cases where the OOS is caused by a batch quality failure (SQUIPP) and it does not meet established standards and specification the batch should be rejected.
- For inconclusive investigations that do not reveal the root cause nor reproduce the OOS the batch disposition is made by the head of QA and a specific batch variation may need considering.
- Any final decision by the head of QA to release a batch which has a proven OOS but does not effect the quality of the batch must be done so with extreme caution and if appropriate with consultation with regulatory authorities (drug shortage, extreme patient needs etc.).

Records of Investigation

A written record of the review should include the following information.

1. A clear statement of the reason for the investigation.
2. A summary of the aspects of the manufacturing process that may have caused the problem.
3. The results of a documentation review, with the assignment of actual or probable cause.
4. The results of a review made to determine if the problem has occurred previously.
5. A description of corrective actions taken.

OOS Documentation Needed

- SOP - predetermines laboratory course of action.
- Standard Forms for Analyst Investigation.
- Written authorised investigation report (s).
- OOS Trend Record / Register
- The FDA guideline states:
  - Investigations along with conclusions reached must be preserved with written documentation that enumerates each step of the investigation. The evaluation, conclusion and corrective action, if any, should be preserved in an investigation or failure report and placed into a central file.

Examples of OOS Regulatory Citations

- No investigation of temperature deviations during stability study
- OOS investigations failed to follow retest procedure
- Concluded that OOS result due to insufficient shaking/ extraction without data or documentation
- OOS results invalidated as caused by improper sample preparation without data documentation
- Manufacturing process/raw materials/batch record history not reviewed as required by retest SOP
- Product released after OOS result using grand average including in and OOS results

Flash Quiz

#	OOS Investigations	Your Selection
1	Which of the following are acceptable, true or false: a) Outlier testing is a good way of filtering HPLC data from long runs. b) A large plate Bioassay of antibiotic has some larger than usual variation of data, the QC Microbiologist has discussed with analyst and compared to results from tests on the same batch of API (different delivery date) and concluded that an outlier test was justified. Approval is obtained from QA as per Micro procedures. c) Outlier testing cannot be used for potentiometric titration results. d) Bioassays are inherently variable and so you can repeat test and resample once before reporting an OOS.	True/False