ISO 17025: Quality Systems FMD diagnosis in AAHL

CSIRO Health and Biosecurity

Transboundary Animal Diseases Mitigation Team

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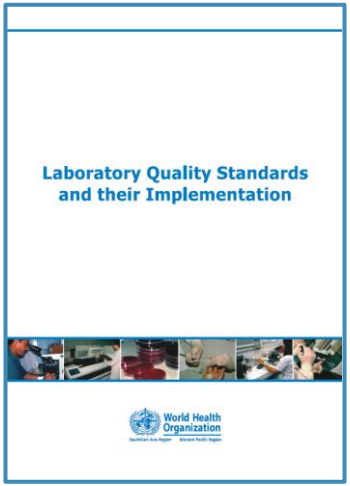
WHO Guidelines – 2011

- Laboratory services are an essential component of quality patient-care delivery (WHO Document)
  - Includes primary health/veterinary care
  - Point-of-care testing



- Quality laboratory results are required to
  - Support clinical diagnosis
  - Rationalize and monitor treatment
  - For surveillance and control of diseases
  - Provide early warning of disease outbreaks
  - Improve accuracy of health information and promote effective national health planning

http://apps.who.int/medicinedocs/documents/s22409en/s22409en.pdf?ua=1

Definitions

- **Quality Control** - QC refers to the measures that must be included during each assay run to verify that the test is working properly.
- **Quality Assurance** - QA is defined as the overall program that ensures that the final results reported by the laboratory are correct.
- **Aim:** To ensure that the results generated by the test are correct
- **However:** QA is concerned with much more.
  - That the right test is carried out on the right specimen,
  - That the right result and right interpretation is delivered to the right person at the right time
- **Quality Assessment** – It is a means to determine the quality of the results generated by the laboratory.
  - Quality assessment is a challenge to the effectiveness of the QA and QC programs.
  - Quality Assessment may be external or internal (PTS)

International laboratory quality standards

- Several internationally accepted standards applicable to laboratories and many of these have been developed by ISO.
- Standards ensure desirable characteristics of products and services such as quality, safety, reliability, efficiency and reproducibility.
- ISO standards are not regulatory and have no legal authority to enforce the implementation of its standards.
- Each country decides which standards fit their situation.

International laboratory quality standards

- A national laboratory plan should specify quality standards for laboratories at each level and define the bodies responsible for establishing, implementing and monitoring those standards.
- For Australia, the National Association of Testing Authorities (NATA) is the authority that provides independent assurance of technical competence.
- NATA provides assessment, accreditation and training services to laboratories and technical facilities

NATA
WORLD RECOGNISED
ACCREDITATION

International standards applicable to laboratories

ISO/IEC 17025	General requirements for the competence of testing and calibration laboratories
ISO 15189	Medical laboratories – particular requirements for quality and competence
ISO/IEC 17043	Conformity assessment – general requirements for proficiency testing
ISO 13528	Statistical methods for use in proficiency testing by interlaboratory comparison
OECD GLP	OECD principles on good laboratory practice
ISO Guide 34	General requirement for the competence of reference material producers
ISO 8402	Quality management and quality assurance – vocabulary
ISO 19011	Guidelines for quality and/or environmental management system auditing
ISO 9001	Quality management systems – requirements

Development and implementation of Quality Systems

- Quality standards are an integral part of the Quality System (QS) and are designed to
  - help laboratories meet regulatory requirements (Local and International)
  - monitor laboratory functions to ensure laboratory safety and consistency in performance

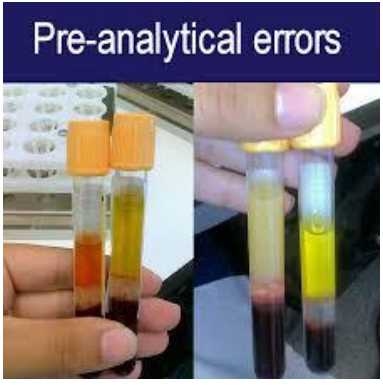
Quality policy	→	Mission statement
Quality plan	→	Implementation of policy
Quality manual	→	Policy, plan and application of standards
Procedures	→	Development and application of SOPs
Work instructions	→	Methodology to carry out specific tasks
Training of staff	→	Implementation of quality system and use of SOPs
Monitoring and evaluation	→	Assessment of quality and correction process

Steps in implementing a QS

- Commitment of top management
- Involvement of all laboratory staff
- Gap analysis (with reference to the selected ISO standards)
- Where to start
  - Technical areas (IQC and EQA)
  - Documentation (SOPs, Assay Validation)
  - Training on the standard
- Audit (Internal and External)
- Management Review
- Continuous quality improvement (CQI)

Managing laboratory specimens – Pre-analytical Phase

- Samples must be collected using appropriate methods and containers, ensuring that there is no spillage of sample or cross contamination.
- Samples must be transported in a safe and secure way as per the requirement for the type of sample collected
- Proper test request forms must be designed and made available
  - For AAHL this is achieved by using the STARS Web service
- Clear identification of samples before it is sent to the laboratory for testing
  - AAHL has a dedicated Samples Reception Team that enter all the information on to LIMS
  - Each sample is identified by a unique Sample Accession Number (SAN) for traceability and reporting.



Managing laboratory specimens – Analytical Phase

- Testing protocols must be designed based on the capacity and capabilities of the facilities , equipment and staff availability and the number of samples submitted for examination.
- Validated SOPs must be made available for all analytical methods.
- There are >110 accredited diagnostic tests implemented in AAHL.
- IQC system must be available to verify that the intended quality of result is achieved for every batch of test. Non compliance must be identified, dealt with appropriately and corrective action must be taken and recorded.

Managing laboratory specimens – Post-analytical Phase

- Designated staff review the test results and authorise release of results (AAHL implements a system of daily interaction of duty veterinarians and diagnosticians on each test result and the IQC data).
- Samples must be stored for a reasonable amount of time and be made available for re-examination
- Occurrence management
  - The laboratory must have a mechanism for staff to document and report problems in laboratory operations which may interfere test results.
  - Appropriate correction action must be planned and implemented for the problems identified, reported and reviewed.

Customer service and resolution of complaints

- The laboratory head and authorized staff must be prepared to offer advice to clinical staff and other customers on the use of the service, including operating hours and emergency samples, the types of samples required and interpretation of the results.
- There should be regular meetings between the laboratory head and the users of the service to discuss ways of improving the working of the laboratory.
- A mechanism should be established to document notification to customers when the laboratory experiences delays or interruptions in testing (due to equipment failure, stock-outs, fall in staff levels, etc.) or finds it necessary to change examination procedures.
- Procedures including documents must be developed for receiving, recording and processing all complaints. Records of complaints, their resolution and minutes of meetings with the users of the service must be recorded and evaluated.

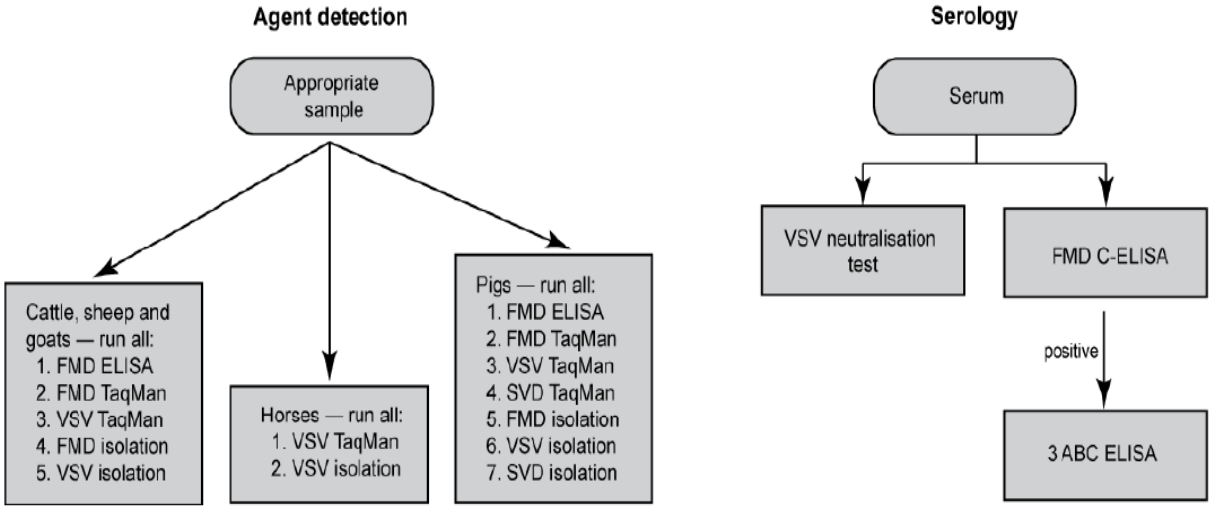
Implementing laboratory quality standards at national level

- National consensus for agreed standards and approval by the appropriate national authorities.
- An implementation plan with short-, medium- and long-term objectives, activities and timelines, and indicative annual budgets.
- Identify an implementing agency and sensitize them to the plan and their possible contributions (Govt / NGO / Private sector).
- Sensitize participating institutions and organisations
- Use or amend existing guidelines and documents or develop country-specific documents.
- Establish national procedures for laboratory networking and referral of samples (LEADRR network in Australia).
- Draw up detailed annual operational plans with budgets.

Implementing laboratory quality standards at laboratory level

- Building leadership and involving all concerned staff in the process.
- Make essential changes
  - Some like reorganisation are easy to implement and cost little.
  - Other changes require moderate inputs and funding
  - Yet other changes are more expensive or more difficult to implement.
- Start by making simple and easy-to-implement changes
- Introduce SOPs for particular procedures or activities one by one.
- Make arrangements to conduct regular meetings with users of the service.
  - This will have the benefit of keeping users informed of the efforts being made to improve the quality of the laboratory service.
- A checklist may be used to establish a baseline of implementation of laboratory quality standards as well as monitoring the progress made.

Laboratory diagnostic algorithm - FMDV



3ABC-ELISA = DIVA test (see Table 1.2); C-ELISA = competition ELISA; ELISA = enzyme-linked immunosorbent assay; FMD = foot-and-mouth disease; SVD = swine vesicular disease; VSV = vesicular stomatitis virus

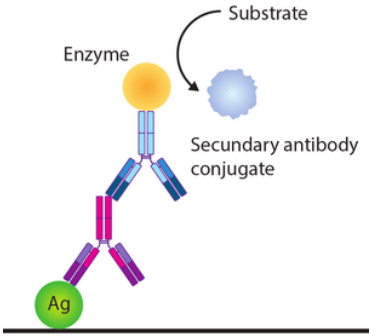
Note: The CSIRO Australian Animal Health Laboratory treats any vesicular disease exclusion by testing for all appropriate vesicular disease: samples submitted for either FMD, VSV or SVD exclusion will be automatically tested for the other relevant vesicular diseases.

Quality Systems – FMD diagnosis in AAHL

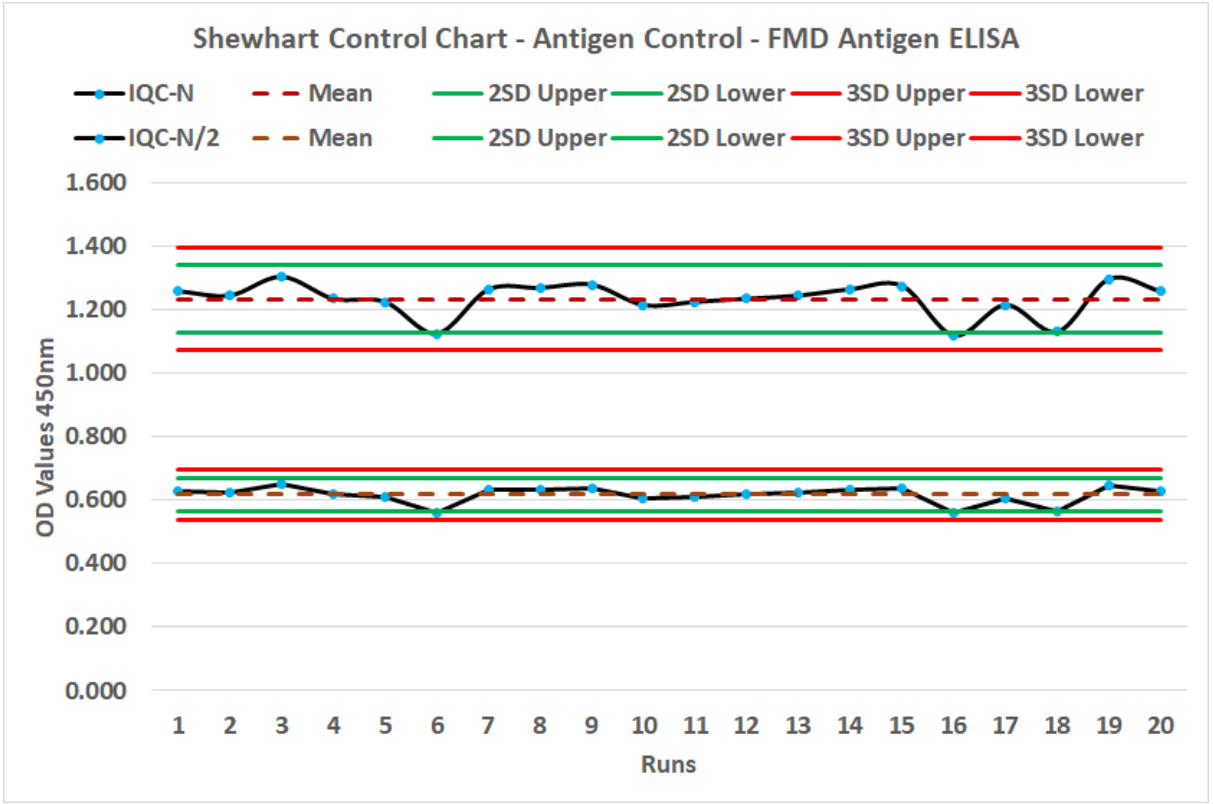
- Antigen Detection
  - ELISA
  - Virus Isolation using cell culture (BTY, BHK-21 & LFBK cells)
- Genome Detection
  - Real-time RT-PCR – Pirbright Protocol (5'-IRES region)
  - Real-time RT-PCR – Tetracore™ Protocol ($3D^{pol}$ region)
  - Multiplex Real-time RT-PCR (combined)
  - Multiplex Real-time RT-PCR (combined with 18S control)
- Antibody Detection
  - NSP 3ABC cELISA (in-house protocol & PrioCHECK FMDV NS ELISA Kit)
  - SPCE for all seven FMDV serotypes
  - LPBE for all seven FMDV serotypes

Internal Quality Standards: Antigen Detection ELISA

- Positive antigens of 7 serotype and a negative control
- Daily monitoring sheet for OD values; plate-to-plate & day-to-day variations
- Establish a mean value and 95% confidence interval (CI)
- Draw progressive monitoring for the OD values (accuracy and precision)
- Acceptance criteria:
  - The positive antigens must have an OD reading greater than 0.8.
  - Background wells should have an OD reading of less than 0.2
  - Negative controls should have OD readings of less than 0.2

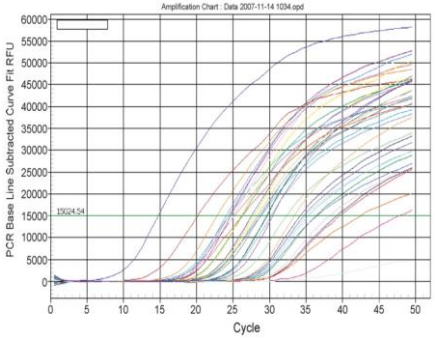


FMD Antigen ELISA – OD value of positive control well (Neat and Neat/2)

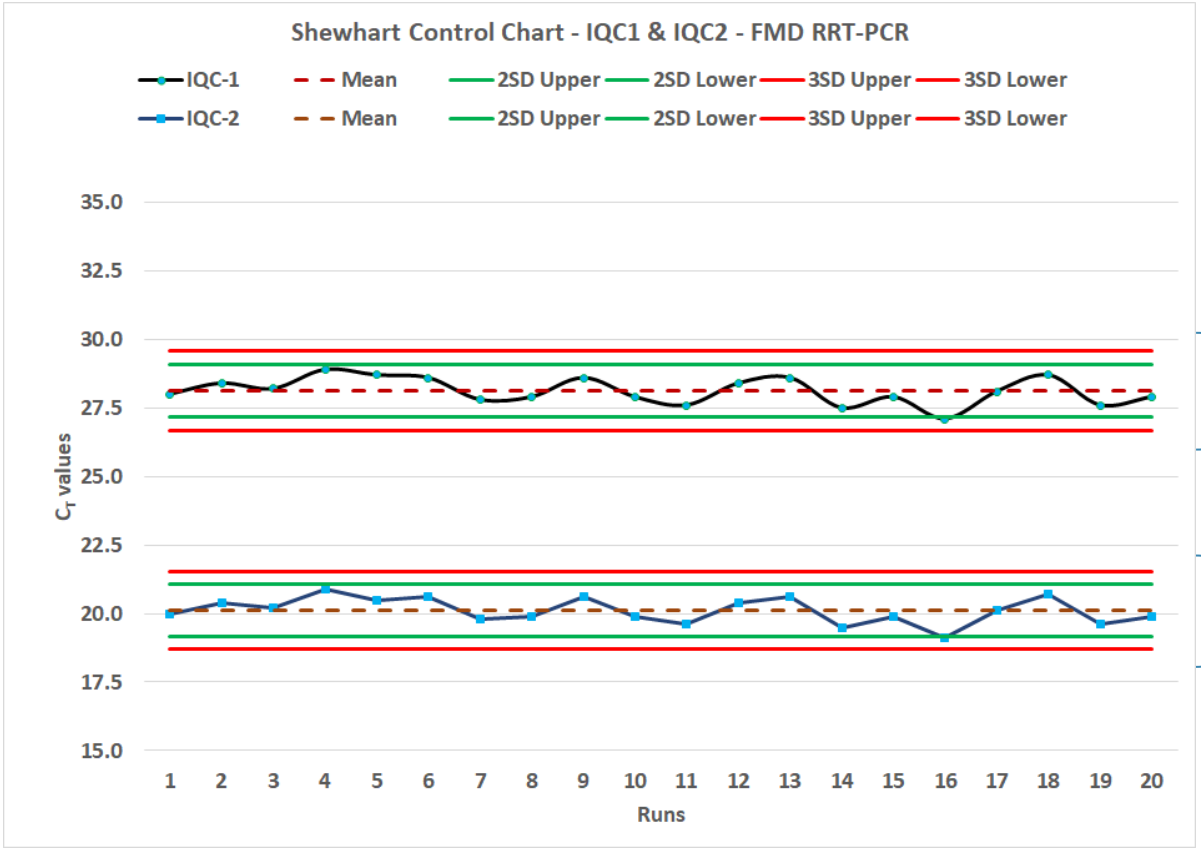


Internal Quality Standards: Genome Detection

- Internal positive control – non replicating Plasmid DNA with FMD genome
- Negative extraction control
- No template control
- 18S RNA control (Reaction control; optional)
- Establish a mean Ct value and 95% CI
  - High positive control
  - Low positive control
- Standardized baseline threshold 0.2
- Draw progressive monitoring for the Ct values (accuracy and precision)

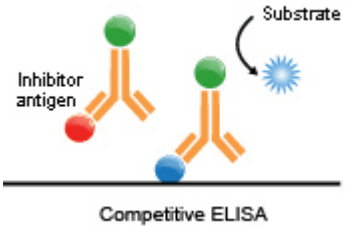


FMD real-time RT-PCR – IQC data



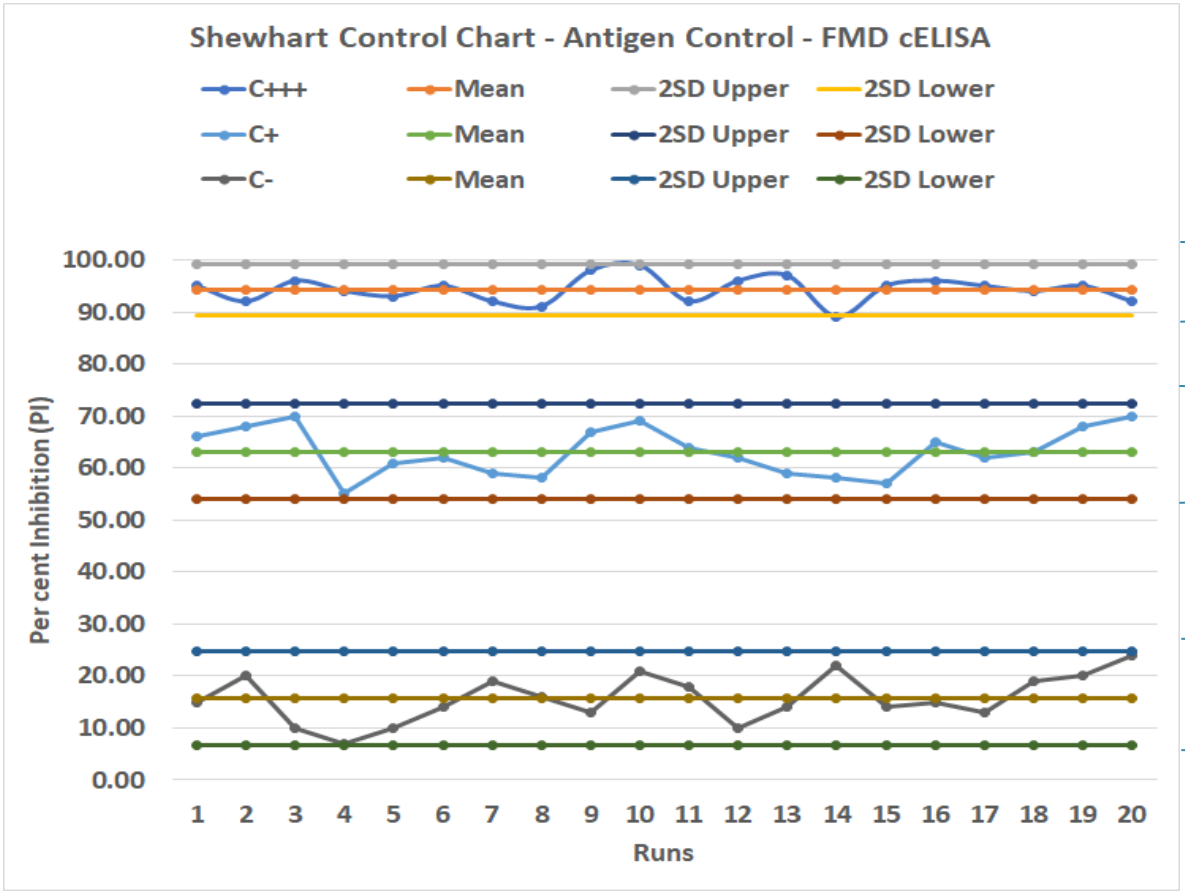
Internal Quality Standards: NSP antibody assays

- Assay controls: High (C+++) and Low (C+) Positive controls; Negative control (C-), Control Antigen (OD Max) and Antibody-Enzyme Conjugate control (CC).
  - Establish a mean per cent inhibition (PI) values, and their respective 95% CI.
- Internal Positive Control (IPC): Run on every assay and the PI value monitored against the mean and 95% CI estimates (Optional)
- Acceptance criteria based on PI value of assay controls
  - CC wells must have OD values <0.2
  - C+++ should be in the range 80 to100%
  - C+ should be in the range 50 to 80%
  - C- should be in the range 0 to 30%
  - Median OD Max value should range between 0.8 to 1.5 and individual PI value of OD Max should be in the range -25 to 25 %
- IPC data used for monitoring assay performance



Competitive ELISA

FMD NSP cELISA / SPCE – PI values



Internal Quality Standards: Serotype specific antibody assays

- Assay controls: High (C+++) and Low (C+) Positive controls; Negative control (C-), Control Antigen (OD Max) and Antibody-Enzyme Conjugate control (CC).
  - Establish a mean per cent inhibition (PI) values, and their respective 95% CI.
- Internal Positive Control (IPC): Run on every assay and the PI value monitored against the mean and 95% CI estimates (optional)
- Acceptance criteria based on PI value of assay controls
  - CC wells must have OD values <0.2
  - C+++ for that serotype should be in the range 80 to100%
  - C+ for that serotype should be in the range 50 to 80%
  - C- should be in the range 0 to 30%
  - Median OD Max value should range between 0.8 to 1.5 and individual PI value of OD Max should be in the range -25 to 25 %
- IPC data used for monitoring assay performance

Variables that affect the quality of results

- The educational background and training of the laboratory personnel
- The condition of the specimens
- The controls used in the test runs
- Reagents
- Equipment
- The interpretation of the results
- The transcription of results
- The reporting of results

Errors in diagnostic testing

- ***Pre-analytical phase***
  - Incorrect test request or test selection
  - Incomplete laboratory request forms
  - Incorrect specimen collection, labelling and transportation

- ***Analytical phase***
  - Use of faulty equipment, improper use of equipment
  - Use of substandard or expired reagents
  - Incorrect reagent preparation and storage
  - Incorrect technical procedures; non-adherence to standard operating procedures (SOPs) or internal quality control (IQC)

- ***Post- analytical phase***
  - Inaccurate reporting and recording
  - Inaccurate calculations, computation or transcription
  - Return of results to the clinician too late to influence patient management
  - Incorrect interpretation of results

Errors in measurement

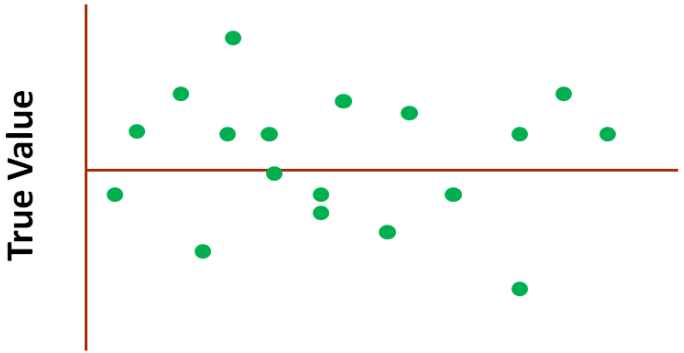
- **True value** - this is an ideal concept which cannot be achieved.
- **Accepted true value** - the value approximating the true value, the difference between the two values is negligible.
- **Error** - the discrepancy between the result of a measurement and the true (or accepted true value).

Sources of error

- Input data required - such as standards used, calibration values, and values of physical constants.
- Inherent characteristics of the quantity being measured - e.g. CFT and HAI titre.
- Instruments used - accuracy, repeatability.
- Observer fallibility - reading errors, blunders, equipment selection, analysis and computation errors.
- Environment - any external influences affecting the measurement.
- Theory assumed - validity of mathematical methods and approximations.

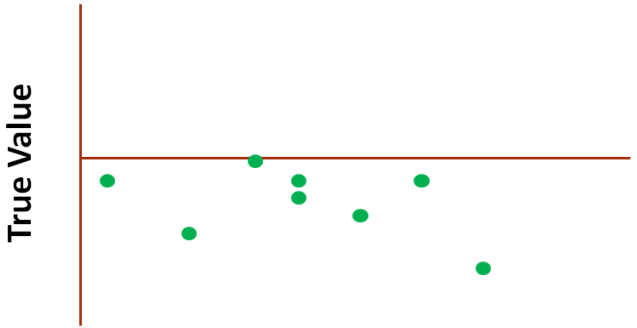
Random Error

- An error which varies in an unpredictable manner, in magnitude and sign, when a large number of measurements of the same quantity are made under effectively identical conditions.
- Random errors create a characteristic spread of results for any test method and cannot be accounted for by applying corrections. Random errors are difficult to eliminate but repetition reduces the influences of random errors.
- Examples of random errors include errors in pipetting and changes in incubation period. Random errors can be minimized by training, supervision and adherence to standard operating procedures.



Systematic Error

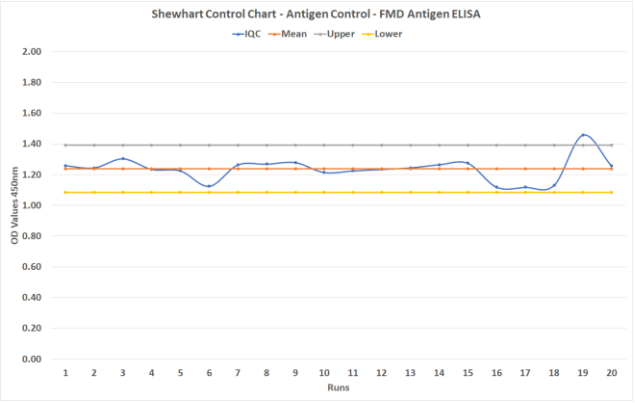
- An error which, in the course of a number of measurements of the same value of a given quantity, remains constant when measurements are made under the same conditions, or varies according to a definite law when conditions change.
- Systematic errors create a characteristic bias in the test results and can be accounted for by applying a correction.
- Systematic errors may be induced by factors such as variations in incubation temperature, blockage of plate washer, change in the reagent batch or modifications in testing method.



Shewhart Control Charts

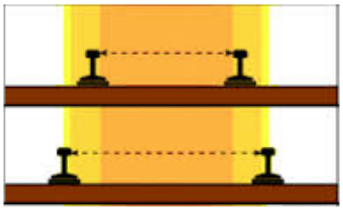
A Shewhart Control Chart depend on the use of IQC specimens and is developed in the following manner:-

- Put up the IQC specimen for at least 20 or more assay runs and record down the O.D./cut-off value or antibody titre (whichever is applicable).
- Calculate the mean and standard deviations (s.d.)
- Make a plot with the assay run on the x-axis, and O.D./cut-off or antibody titre on the y axis.
- Draw the following lines across the y-axis: mean, upper and loser limits of 3 and 2 SD.
- Plot the O.D./cut-off obtained for the IQC specimen for subsequent assay runs.
- Major events such as changes in the batch no. of the kit and instruments used should be recorded on the chart.



Westgard rules

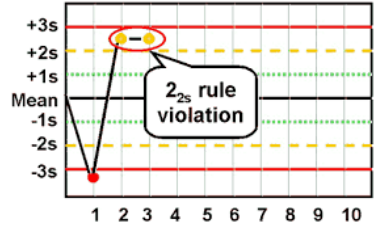
- Analyze data in Shewhart control charts.
- Define specific performance limits for a particular assay
- Detect both random and systematic errors.
- There are six commonly used Westgard rules
  - Three are warning rules
  - Three are mandatory rules
- The violation of warning rules should trigger a review of test procedures, reagent performance and equipment calibration.
- The violation of mandatory rules should result in the rejection of the results obtained with patients' serum samples in that assay.



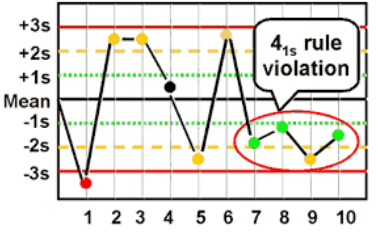
Westgard's Warning rules

- Warning $\mathbf{1_{2SD}}$: It is violated if the IQC value exceeds the mean by ±2SD. It is an event likely to occur normally in less than 5% of cases.

- Warning $\mathbf{2_{2SD}}$: It detects systematic errors and is violated when two consecutive IQC values exceed the mean on the same side of the mean by ±2SD.

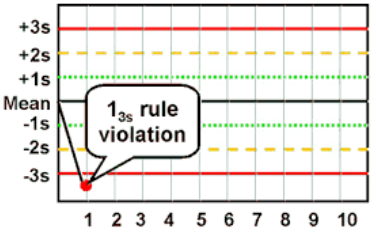


- Warning $\mathbf{4_{1SD}}$: It is violated if four consecutive IQC values exceed the same limit (mean ± 1SD) and this may indicate the need to perform instrument maintenance or reagent calibration.

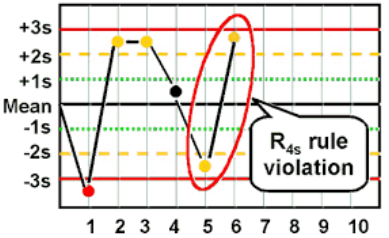


Westgard's Mandatory rules

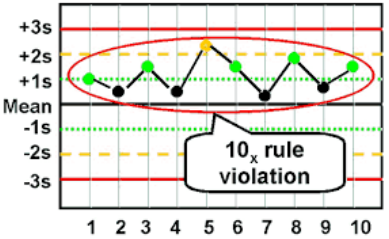
- Mandatory **1_{3SD}** : It is violated when the IQC value exceeds the mean by ±3SD. The assay run is regarded as out of control.



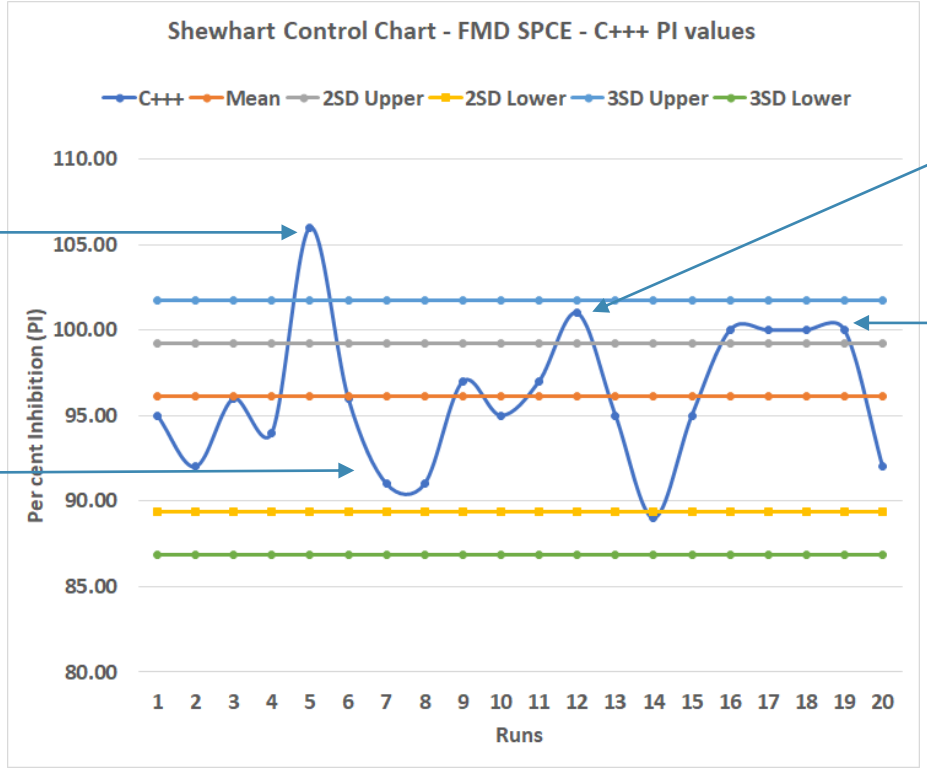
- Mandatory **R_{4SD}** : It is only applied when the IQC is tested in duplicate. This rule is violated when the difference in SD between the duplicates exceeds 4SD.



- Mandatory **10x** : This rule is violated when the last 10 consecutive IQC values are on the same side of the mean or target value.



Westgard rules: Warning and Mandatory rule infringements



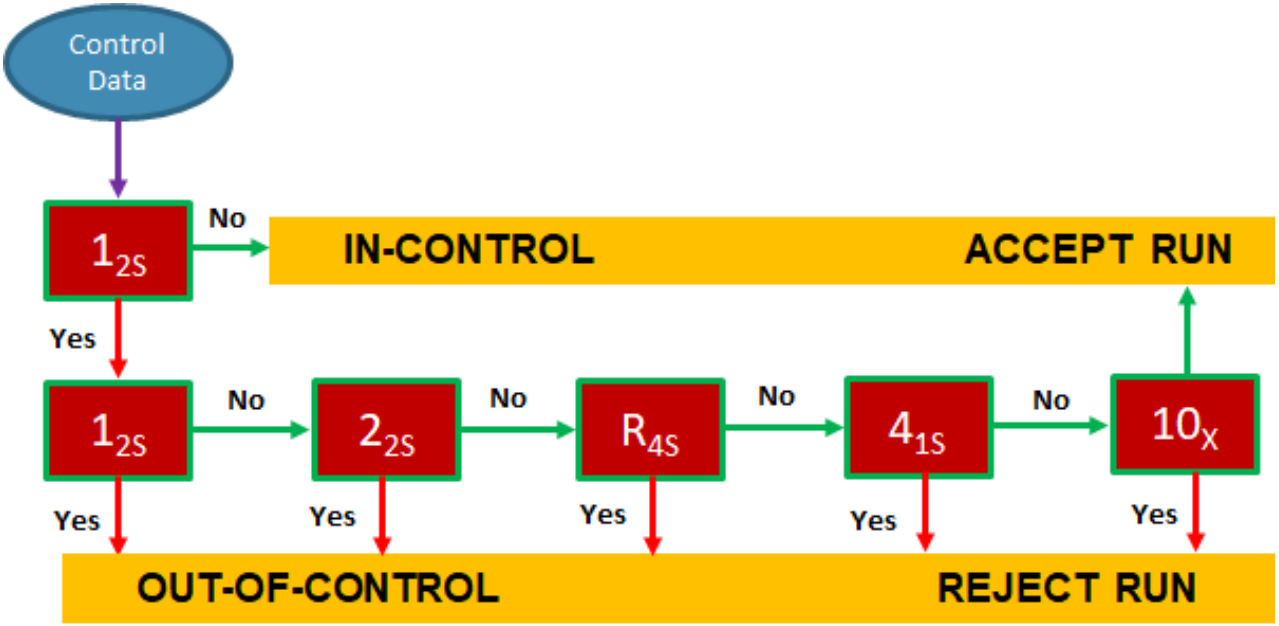
$M1_{3SD}$

$W1_{2SD}$

$W2_{2SD}$

$W4_{3SD}$

Westgard Rules and multi-rules – Interpretations



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IMPROVED SURVEILLANCE, PREPAREDNESS AND RETURN TO TRADE FOR EMERGENCY ANIMAL DISEASE INCURSIONS USING FOOD AND MOUTH DISEASE AS A MODEL

PROJECT PARTNERS

CSIRO

Australian Government
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MEAT & LIVESTOCK AUSTRALIA

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Australian Government
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ALFA
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AUSTRALIAN Pork

CATTLE COUNCIL OF AUSTRALIA

animalhealth AUSTRALIA

GICA

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