Quality issues in Laboratories dealing with FMDV

Background to laboratory quality assessment
Quality systems, established to internationally accepted standards, are one mechanism that can assist in evaluations of the:
1) Sustainability of technology transfer
2) Proficiency of the user
3) Reliability and comparability of data generated, resulting in potential enhancement of laboratory credibility.

The development and adaptation of immunoassay and molecular techniques for use in national veterinary laboratories in developing countries, the standardization of equipment, working protocols, and interpretation of test results, and the evaluation of assay reliability under the conditions found in such laboratories (validation) are complex and time-consuming activities.

The situation is complicated by the number and diversity of diagnostic kits used, the variability of working conditions and staff expertise at laboratories in developing countries, and the stressful conditions that the kit components suffer in transit to counterpart laboratories.

Ministries of Agriculture determine policy, make decisions and take actions affecting the livestock sector, based in part on information provided by a national veterinary laboratory.
However few developed or developing countries have mechanisms for recognition of quality management (QM) or technical competence of these laboratories.

Quality issues in labs dealing with FMDV

Roles of laboratories

- Receiving and testing samples for confirmation of clinical diagnosis of FMD
- Testing samples to differentiate FMDV from other viruses
- Receiving serum samples to examine for antibodies against FMDV
- Analysing data from tests
- Providing information and advice to those involved in management of disease
- Storage and maintenance of serum and antigen banks
- Providing expertise to other laboratories
- Training scientists in diagnostic techniques

The exact roles of individual laboratories will depend on the plans formulated. As already stated this can be simple, where there are few facilities to extremely complex

An emerging challenge, in part in response to the World Trade Organization (WTO) Sanitary Phytosanitary Agreement, is to make the organization and operations of national-level veterinary services, including diagnostic testing laboratories, clear to outside observers so that the quality and comparability of animal health programmes and data can be evaluated. Historically, the Organisation International des Epizooties (OIE) has been a focus of information gathering and dissemination for animal health trade issues.

Following the completion of the Uruguay round of General Agreement of Trade and Tariffs (GATT) and formation of the World Trade Organisation (WTO) the OIE was given the task of continuing this process of livestock trade facilitation. Unfortunately there has never been an agreed mechanism for assessing the value or reliability of the data reported to the OIE, but as international and regional trade agreements become established, the need for international standards of QM and technical competence for veterinary diagnostic testing laboratories, and a common method for monitoring compliance with these standards has never been greater.

A variety of processes have been developed worldwide to recognize quality assurance (QA) systems in the manufacturing, production, and service sectors, as well as the technical competence of testing laboratories. In particular, the ISO/IEC 17025 forms the basis of many national standards for recognition of the competence of calibration and testing laboratories while the OECD Principles of Good Laboratory Practice (OECD-GLP) are used internationally as a basis for determining a laboratory's compliance with safety study guidelines. It is noted that some Government laboratories in developed countries have achieved both the equivalent of ISO 17025 Accreditation and OECD Compliance recognition

Quality issues in labs dealing with FMDV

Basic parameters

- Good standard waterproof buildings
- Negative pressure facilities
- Cell cultures
- Cabinets for cell cultures
- Safety cabinets to prevent virus dissemination
- Equipment vital to tests
- Reagents (kits, chemical etc) for tests
- Molecular biological equipment and materials
- PCR set up including separate laboratories
- Plastic vessels for cell culture
- Medium for cell culture
- Distilled water and deionised water
- Low temperature freezers/refrigerators
- Constant power and standby generator with fuel
- Quality control in place for all tests
- Well-trained knowledgeable staff
- Good laboratory practice in place and well managed
- Laboratory accreditation

A laboratory has to have good buildings that are safe. They should resist weather to avoid damage and be secure in terms of entry. Negative pressure facilities are vital especially where large amounts of virus is produced e.g. in animal experiments. The tests used demand great resources and conditions. A full diagnostic service needs reagents (kits) and technologies with expensive equipment. Plastics alone for tissue culture can be very costly. The management of tests, data, people and reporting is difficult and training on a continuous basis is necessary. Eventually the status of a laboratory could determine trade issues and accreditation may be needed to assure results.

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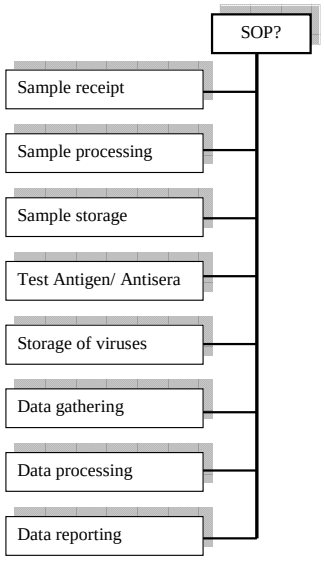
Laboratory tasks



- General Questions
- Pipettes calibrated?
- PH buffers checked?
- Laminar flow cabinets routinely calibrated?
- Distilled water checked?
- Refrigerators and freezers checked and monitored?
- Instrument checked?
- All results recorded
- Sample identity system?
- Sample data base?
- Training continuous?
- Testing of staff?
- Internal quality control?
- External quality control?
- Proficiency testing?
- Reserve power?
- Quality manual?

Here is a chart examining the duties of a laboratory. Some questions to be asked are shown which hints at the depth of work needed to maintain a laboratory. In essence good data has to be produced and shown to be transparent. This means that tests have to be made with approved methods with quality control of all aspects to give confidence that data is defined statistically to acceptable limits. Note internal and external control features are highlighted.

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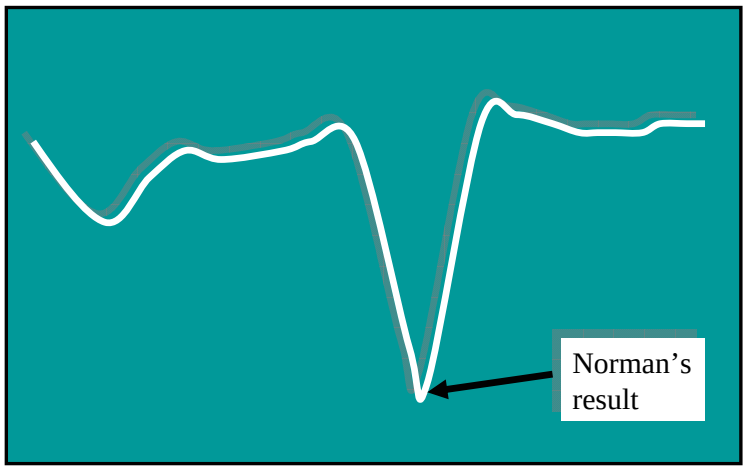
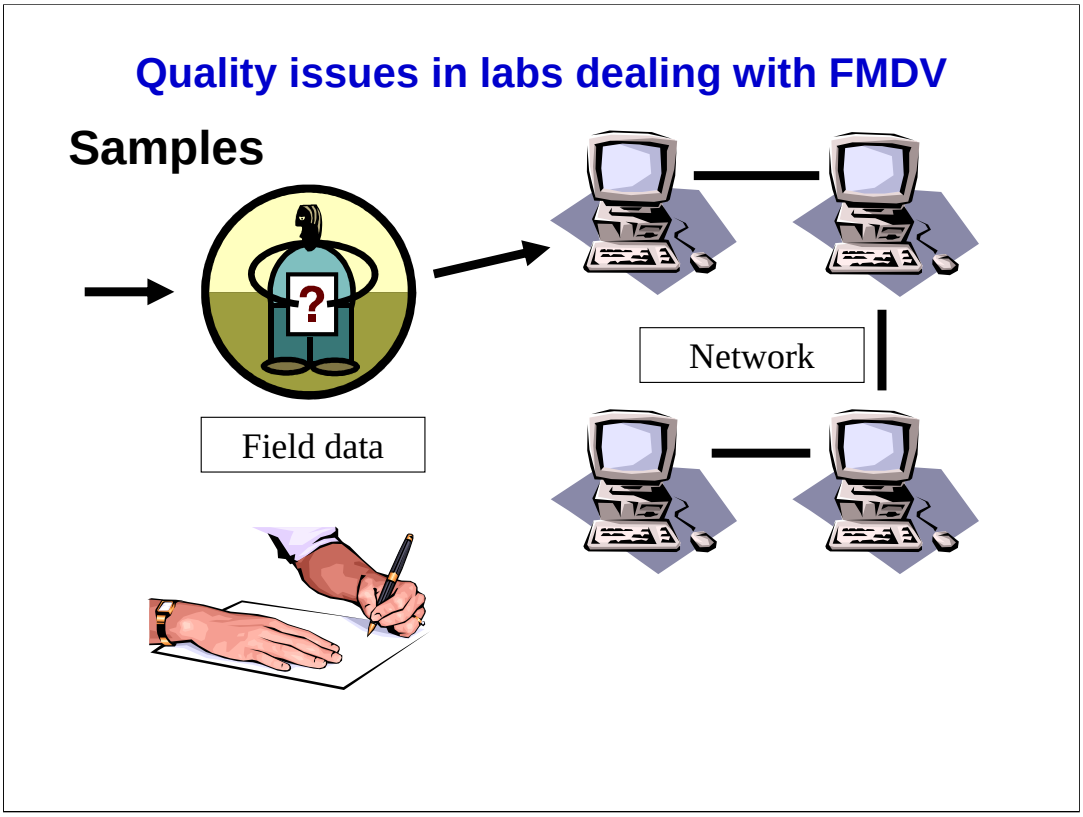
Internal Quality Control (IQC)

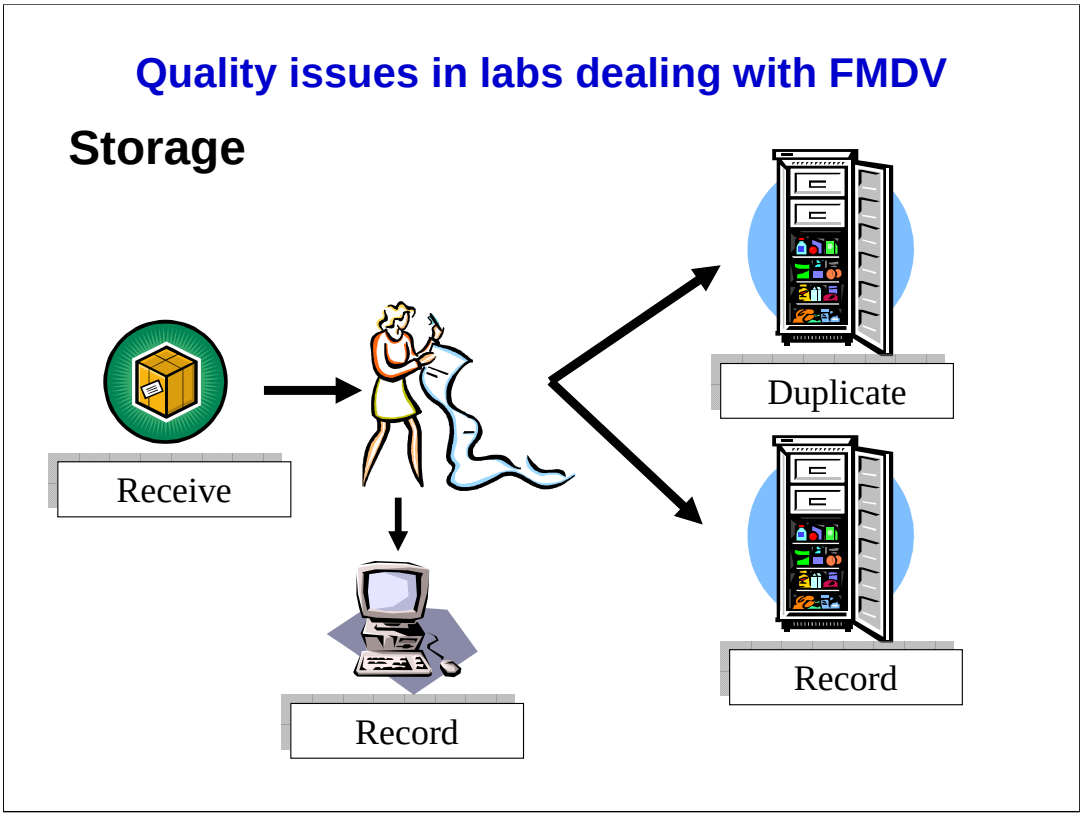


This can be defined as the set of procedures undertaken by the staff of a laboratory for continuously assessing laboratory work and the emergent results, in order to decide whether they are reliable enough to be released (either in support of clinical decision making or for epidemiological or research purposes). Thus quality control procedures have an immediate effect on the laboratory's activities and should actually control, as opposed to merely examining the laboratory's output.



Samples
All sample information should be stored on a secure computer database system protected by restricted access and passwords. Computer systems should ideally be networked and backed-up on a daily basis. Back-up copies should be stored in a fireproof safe with a second back-up copy located off-site. The use of staff dedicated to the maintenance and back-up of the database is strongly advised. Laboratories should take measures to ensure that the correct sample referral details are transferred on to the computer. A checking procedure should be used and the transferred information verified by a suitably qualified staff member.

Laboratories should have a procedure for dealing with samples with incomplete or incorrect data. The laboratory should not analyse this type of sample. The responsibility lies with the referring clinician to provide the correct information. It is advisable that the laboratory keeps a copy of the information as documented proof. Sample tubes accompanying a referral should have at least two pieces of unique identification to link them together (such as a barcode, name, age). Manual transfer of information is always liable to error. The less manual transfer of information the better. Printed sample numbers should be used where possible. Ideally, printed labels with all the details should be generated by computer at the time of booking a sample in. If information in permanent records is altered for any reason then an explanation for the change should be inserted and initialled by the person making the changes to it.



Storage

Storage temperatures should be suitable for maintaining the integrity of the virus over time.
Care should be taken to minimise the number of freeze thaws a sample undergoes as this may compromise quality. Laboratories may find it convenient to take a working aliquot to store at 4^{o}C while testing is undertaken. A duplicate back-up sample should be stored before and/or after manipulation. Duplicates should be stored for a minimum period of 1 year in a second freezer, preferably in another department or building. This is beyond the means of many laboratories. The labels on stored samples should include at least two pieces of identifying information. It is always worth remembering that numbers are more susceptible to transposition errors than words.

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Contamination

Outside — Air and through staff to affect animals

Inside — Contamination of samples and staff

Contamination

Laboratories should take steps to minimise the risks of contamination. Checking for contamination and also ensuring that all equipment is in full working order. Training is aseptic techniques is vital.

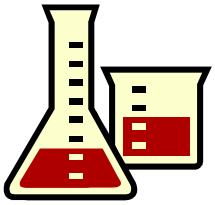
Sample handling

Records should be kept of the batch numbers of all laboratory solutions for traceability and troubleshooting. Ready-made solutions from commercial manufacturers are recommended although the costs involved may mean that some laboratories prefer to use "in-house" reagents. Laboratories should have a procedure in place to ensure that the correct sample is examined.

Examples would include the labelling of each tube with a unique code written on both the lid and side and the checking of tube codes before, during and after transfers with verification / signing off by a suitably qualified member of staff.

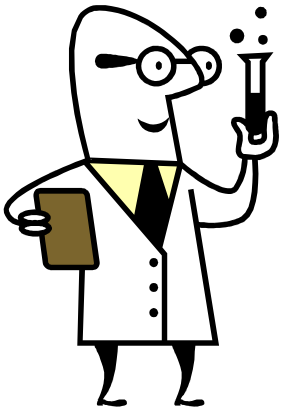
Quality issues in labs dealing with FMDV

Controls



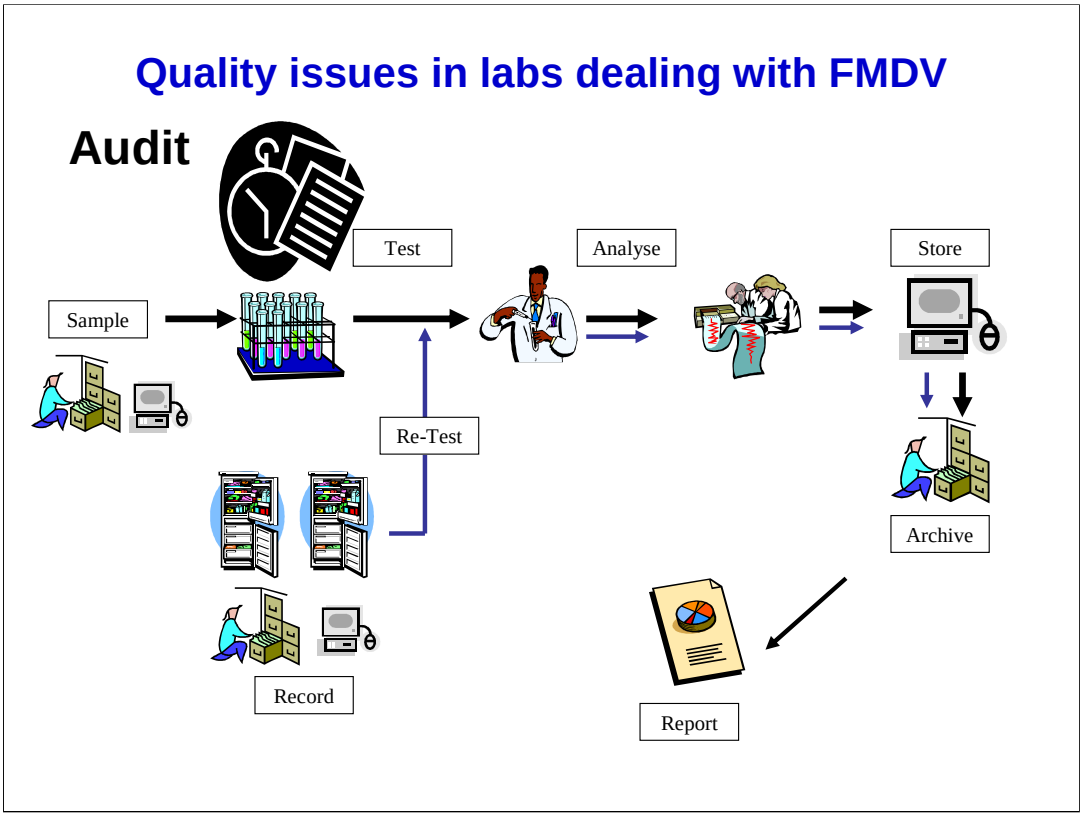
Always include

Reference standards where possible



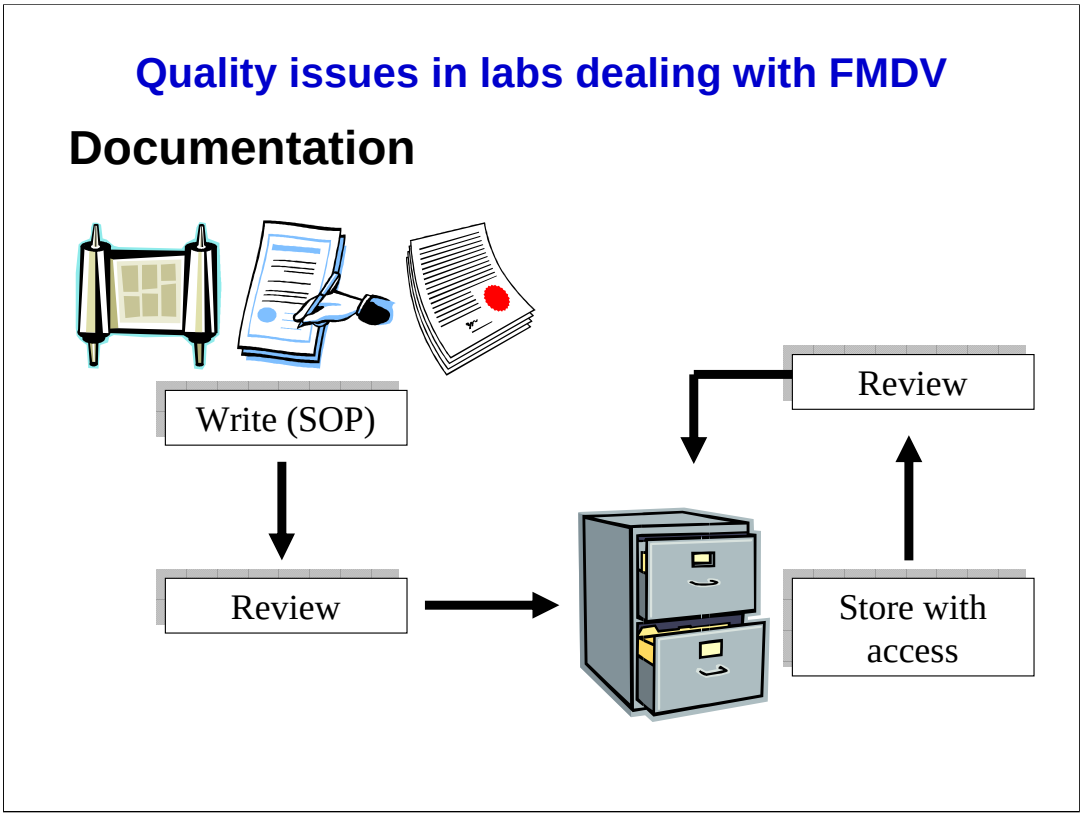
Controls

Control samples, molecular weight markers, negative controls etc., should always be used in diagnostic tests as appropriate. Controls have to be included in all kits to allow relative measurements of samples to be made. Controls have to be stored and used as instructed since if they lose activity the whole test results for samples will be affected.



Audit

Laboratories should have a procedure that enables the following of an audit trail on a sample allowing a suitably qualified member of staff to trace all available information on this, including the original sample(s), raw data and final report.

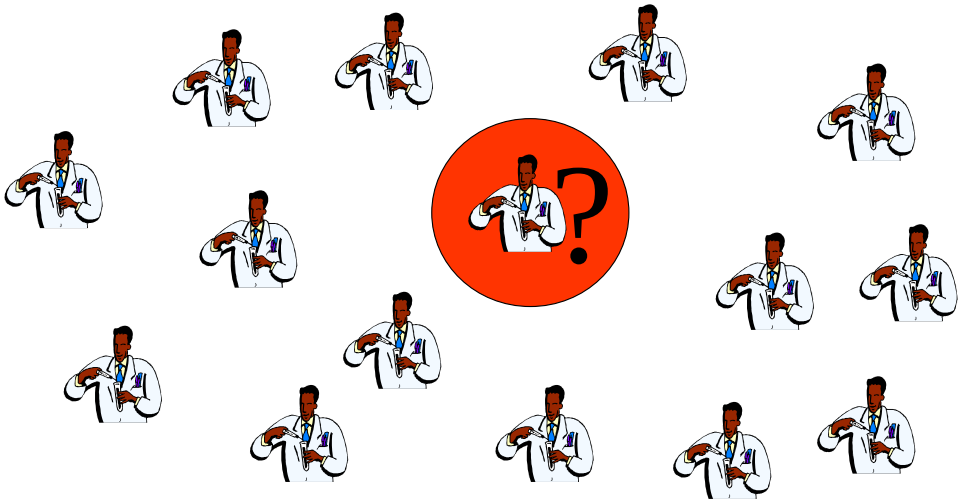


Documentation

Every laboratory should have a collection of standard laboratory protocols for all the techniques used in diagnostic tests. Laboratories should have a documented procedure in place to ensure that only the most up to date copies of laboratory documentation are available to staff. The procedure should include a system for periodic review and/or revision of documents, the notification to staff of the withdrawal of old documents, and archiving of all old documentation. Laboratories may wish to consider the use of a computer database to assist document control.

Quality issues in labs dealing with FMDV

Validation



Laboratories should validate all their diagnostic tests to ensure that they meet acceptable performance standards and are fit for the purpose for which they will be used

Quality issues in labs dealing with FMDV

Training of staff

Education

Skills/knowledge

Data management

Safety

Performance

Staff employed in diagnostic laboratories should be suitably qualified and specifically trained in the methods used for particular disorders. Ongoing safety training is also important.

Staff should be encouraged to continuously update their knowledge by reading the current literature and attending appropriate seminars and conferences. It is useful to keep a record of such continuing education for each member of staff.

Quality issues in labs dealing with FMDV

Quality assurance and quality management

The goal of QA – through application of Quality Control (QC) measures – is to establish a system for routine laboratory procedures that copes with the quality requirements and alerts the technician when the systems fails to meet the requirements

In other words - is the test working?

Quality Assurance (QA) and Total Quality Management (TQM)

Classical tools of QA include calibration of instruments, assay validation, intra-run and inter-run controls, internal and external quality controls. Approaches now try and integrate these into a total quality management (TQM) scheme that includes pre-analytic and reporting methods as well as preparatory and common laboratory procedures. The goal of TQM is the (international) harmonisation of laboratory performance and improved cost effectiveness. The ISO 9000 series standards issued by the International Organisation for Standardisation serves as guideline for implementation of TQM and provide a basis for laboratory certification.

Quality is relative and requires external goals and standards for definition and assessment. Quality has been defined as fitness for the intended use.

Quality critically depends on the precision/accuracy of the method BUT always refers to clinical and decision-making requirements. Those requirements need to be translated into laboratory QA.

The total error budget refers to the maximal allowable laboratory error from the clinical or decision-making point of view. It includes biological (within-subject) variation, systematic and random errors.

The goal of QA – through application of Quality Control (QC) measures – is to establish a system for routine laboratory procedures that copes with the quality requirements and alerts the technician when the systems fails to meet the requirements.

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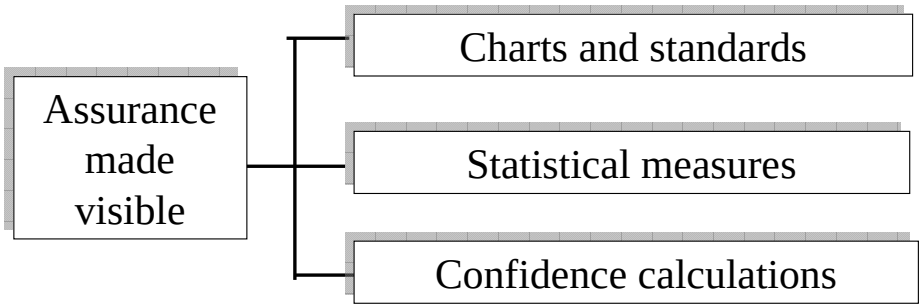
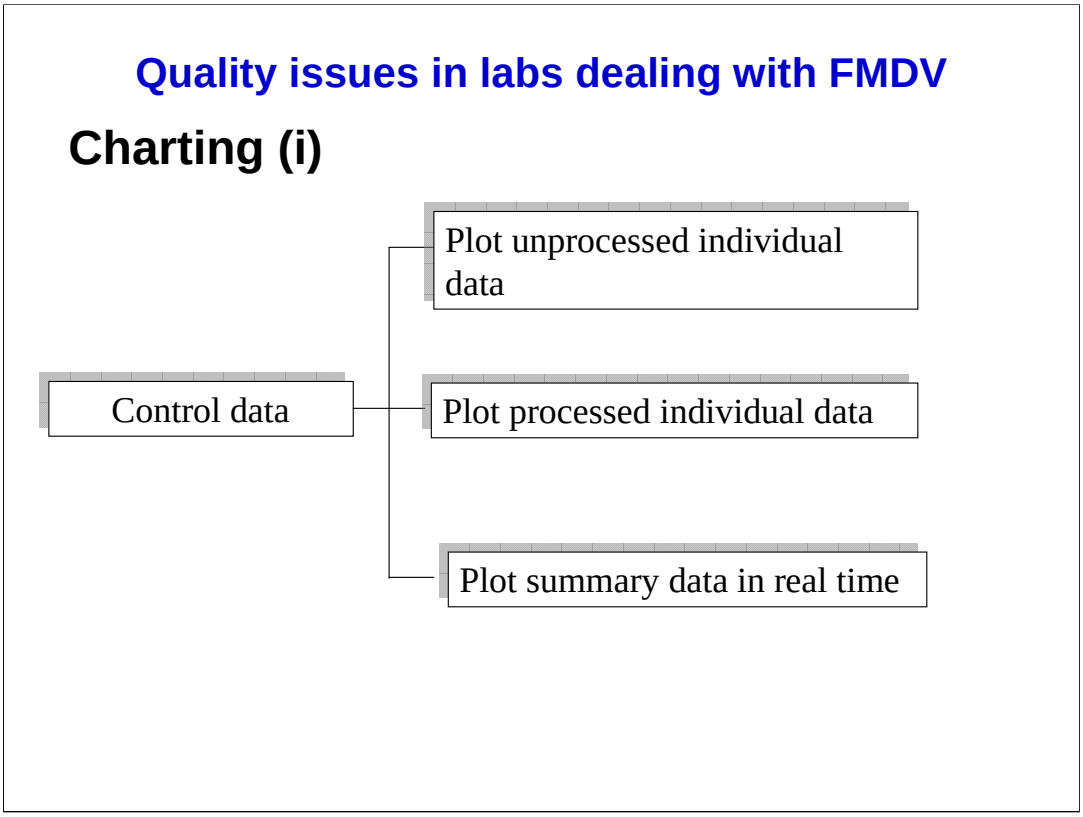
Tools of QA



Quality Control (QC) is the technical realisation of the QA concept.
Controls, control charts and control limits are important elements of QC. These methods were first developed in clinical chemistry but are now also in use for immunodiagnostic methods. Calibration of working standards (in-house internal reagents; denoted as tertiary standards) against National Standard Reagents (National Reference Preparations or secondary standards, which are calibrated against international standards, i.e., primary standards) or inclusion of standards supplied with commercial test kits.
Inclusion of samples for QC (controls) in each run of an assay. Controls are usually calibrated against standards. Rules for the validation of single samples (usually based on their coefficient of variation) and series (usually based on the deviation from the expected value of internal controls).
Control charts (e.g., Levey-Jennings control chart).
Control limits. Calibration of working standards (in-house internal reagents; denoted as tertiary standards) against National Standard Reagents (National Reference Preparations or secondary standards, which are calibrated against international standards, i.e., primary standards) or inclusion of standards supplied with commercial test kits
Inclusion of samples for QC in each run of an assay. QC Controls are usually calibrated against standards Rules for the validation of single samples (usually based on their coefficient of variation) and series (usually based on the deviation from the expected value of internal controls)



Where there is more than one operator charting methods produce a unification of approach to allow control over results and allows discrepancies between performance to be identified. It also promotes the idea of “open” results that can be viewed by anyone, including outside scientists interested in evaluating the status of a laboratory involved in providing results on which management decisions concerning disease control are made.

The most important feature of testing in a laboratory is that operators have a very good understanding of the principles of the test they are making and that they fully understand the nature of their results and the need to process data. There is no substitute for this understanding but the charting method recommended is an aid to simplifying the process of test performance. This section will illustrate methods of keeping a complete picture of any tests made in the laboratory.

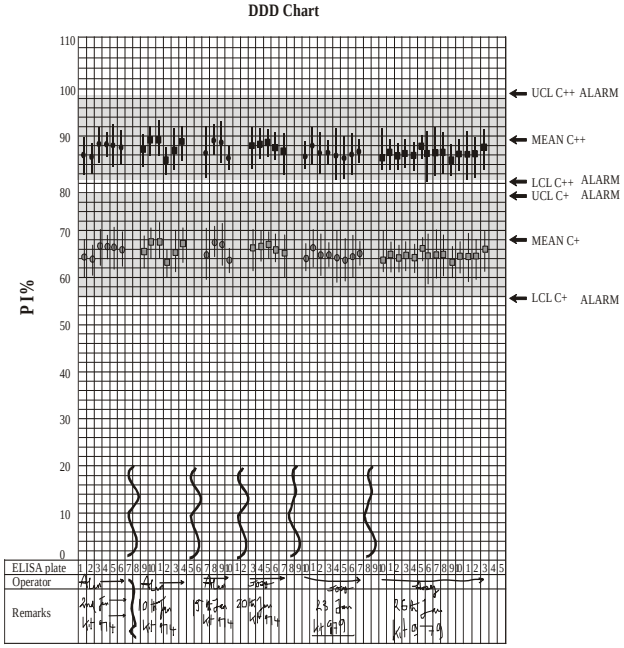
The use of charts is a relatively transparent way of showing all data. Such charts can be displayed openly so that all people involved in running tests can see the day to day, week to week and year to year progress of tests. The charts also indicate whether tests are running well or whether there are problems that should be dealt with.

Quality issues in labs dealing with FMDV

Charting (ii)

ELISA Example
Control positive plots of strong C++ and weaker C+ controls

All data plotted for different tests



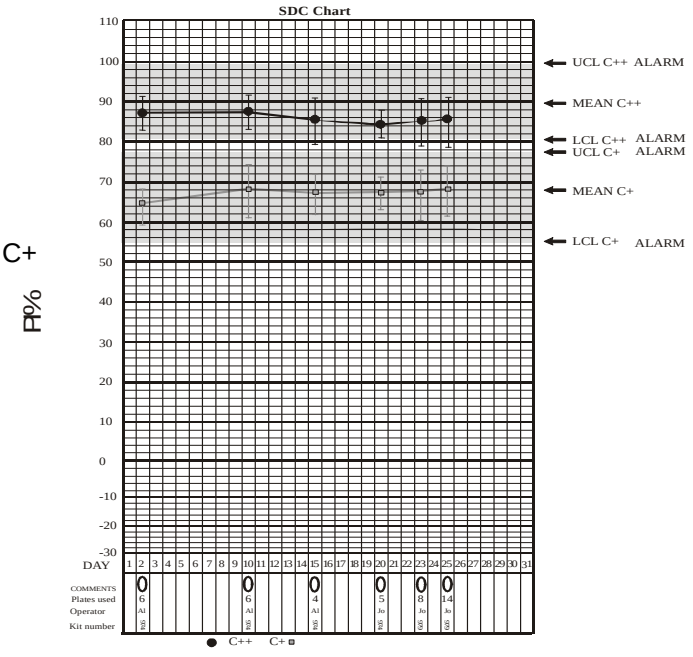
This slide gives an example of ELISA data from a kit. The Control ++ and control C+ are included in kit and run on every plate. The chart shows results of 5 days of testing at different times and with different operators. The data for the controls from every test ELISA plate is show as a percentage inhibition (PI%). Value. The range of acceptance set by the supplier of the kit is shown for the C++ and C+ by the values on the right and in the grey zones. So all the mean values for the PI% of the controls fit between the limits and all the plates show similar results. The data are good on all days and for all operators. If the data was outside these limits, an alarm is noted so that doubts on the test have to be expressed. The controls are being used as Internal Quality Controls.

Quality issues in labs dealing with FMDV

Charting (iii)

ELISA Example
Control positive plots of strong C++ and weaker C+ controls

Data from tests summarised



This slide shows the same data as in the previous slide except that the data for each test day are pooled so that a mean value for the PI% for each control is obtained with a standard deviation value showing the variation in result. This summary data allows control values to be assessed over time to see whether the test is performing similarly from day to day and for all operators.