6. Process control— introduction to quality control

6-1: Introduction

Process control is an essential element of the quality management system, and refers to control of the activities employed in the handling of samples and examination processes in order to ensure accurate and reliable testing. Sample management, discussed in Chapter 5, and all quality control (QC) processes are a part of process control.

QC monitors activities related to the examination (analytic) phase of testing. The goal of QC is to detect, evaluate, nd correct errors due to test system failure, environmental conditions or operator performance, before patient results are reported.

Organization	Personnel	Equipment
Purchasing and inventory	Process control	Information management
Documents and records	Occurrence management	Assessment
Process improvement	Customer service	Facilities and safety

What is QC?

QC is the part of quality management focused on fulfilling quality requirements (ISO 9000:2000 [3.2.10]). Simply put, it is examining "control" materials of known substances along with patient samples to monitor the accuracy and precision of the complete analytic process. QC is required for accreditation purposes.

In 1981, the World Health Organization (WHO) used the term "internal quality control" (IQC), which it defined as "a set of procedures for continuously assessing laboratory work and the emergent results". The terms QC and IQC are sometimes used interchangeably; cultural setting and country may influence preferences for these terms.

In the past few years, "internal quality control' has become confusing in some settings because of the different meanings that have been associated with the term. Some manufacturers of test kits for qualitative tests have integrated "built-in" controls in the design of their kits, which they sometimes refer to as internal controls. Other manufacturers include their own control materials with the kits they sell and they refer to these as "internal controls", meaning that the materials are meant specifically for that manufacturer's kit. Finally, some people refer to any quality control materials that are used in conjunction with test runs as IQC, as in the 1981 WHO definition.

To avoid confusion, the term "quality control" will be used here to mean use of control materials to monitor the accuracy and precision of all the processes associated with the examination (analytic) phase of testing.

QC for varying methods

Quality control processes vary, depending on whether the laboratory examinations use methods that produce quantitative, qualitative or semiquantitative results. These examinations differ in the following ways.

Quantitative examinations measure the quantity of an analyte present in the sample, and measurements need to be accurate and precise. The measurement produces a numeric value as an end-point, expressed in a particular unit of measurement. For example, the result of a blood glucose test might be reported as 5 mg/dL.

Qualitative examinations are those that measure the presence or absence of a substance, or evaluate cellular characteristics such as morphology. The results are not expressed in numerical terms, but in qualitative terms such as "positive" or "negative"; "reactive" or "nonreactive"; "normal" or "abnormal"; and "growth" or "no growth". Examples of qualitative examinations include microscopic examinations, serologic procedures for presence or absence of antigens and antibodies, and many microbiological procedures.

Semiquantitative examinations are similar to qualitative examinations, in that the results are not expressed in quantitative terms. The difference is that results of these tests are expressed as an **estimate** of how much of the measured substance is present. Results might be expressed in terms such as "trace amount", "moderate amount", or "1+, 2+, or 3+". Examples are urine dipsticks, tablet tests for ketones and some serologic agglutination procedures. In the case of other serologic testing, the result is often expressed as a titre—again involving a number but providing an estimate, rather than an exact amount of the quantity present.

Some microscopic examinations are considered semiquantitative because results are reported as estimates of the number of cells seen per low-power field or high-power field. For example, a urine microscopic examination might report 0–5 red blood cells seen per high-power field.

Because QC processes differ for these various types of examinations, the presentations for QC will be divided into two chapters. Chapter 7 will address QC for quantitative examinations, and Chapter 8 will address QC for qualitative and semiquantitative examinations.

Elements of a QC programme

Regardless of the type of examination that is performed, steps for implementing and maintaining a QC programme include:
• establishing written policies and procedures, including corrective actions
• training all laboratory staff
• ensuring complete documentation
• reviewing quality control data.

These responsibilities will be described in more detail in Chapters 7 and 8.

Summary

• QC is part of the quality management system and is used to monitor the examination (analytic) phase of testing.
• The goal of QC is to detect, evaluate and correct errors due to test system failure, environmental conditions, or operator performance, before patient results are reported.
• Different QC processes are applied to monitor quantitative, qualitative and semiquantitative tests.

7. Process control— quality control for quantitative tests

7-1: Overview

Role in quality management system

Quality control (QC) is a component of process control, and is an essential element of the quality management system. It monitors the processes related to the examination phase of testing and allows for detecting errors in the testing system. These errors may be due to test system failure, adverse environmental conditions or operator performance. QC gives the laboratory confidence that test results are accurate and reliable before patient results are reported.

This chapter explains how quality control methods are applied to quantitative laboratory examinations.

Overview of the process

Quantitative tests measure the quantity of a substance in a sample, yielding a numeric result. For example, the quantitative test for blood glucose can give a result of 5 mg/dL. Since quantitative tests have numeric values, statistical tests can be applied to the results of QC material to differentiate between test runs that are "in control" and "out of control". This is done by calculating acceptable limits for control material, then testing the control with the patient's samples to see if it falls within established limits.

As a part of the quality management system, the laboratory must establish a QC programme for all quantitative tests. Evaluating each test run in this way allows the laboratory to determine if patient results are accurate and reliable.

Implementation process

The steps for implementing a QC programme are:
• establish policies and procedures
• assign responsibility for monitoring and reviewing
• train all staff in how to properly follow policies and procedures
• select good QC material
• establish control ranges for the selected material
• develop graphs to plot control values—these are called Levey–Jennings charts
• establish a system for monitoring control values
• take immediate corrective action if needed
• maintain records of QC results and any corrective actions taken.

7-2: Control materials

Defining control materials

Controls are substances that contain an established amount of the substance being tested—the analyte. Controls are tested at the same time and in the same way as patient samples. The purpose of the control is to validate the reliability of the test system and evaluate the operator's performance and environmental conditions that might impact results.

Differentiating controls and calibrators

It is important not to confuse calibrators and control materials. Calibrators are solutions with a specified defined concentration that are used to set or calibrate an instrument, kit, or system before testing is begun. Calibrators are often provided by the manufacturer of an instrument. They should not be used as controls since they are used to set the instrument. Calibrators are sometimes called standards, but the term calibrator is preferred. They usually do not have the same consistency as patients' samples.

Characteristics of control materials

It is critical to select the appropriate control materials. Some important characteristics to consider when making the selection are:
• Controls must be appropriate for the targeted diagnostic test—the substance being measured in the test must be present in the control in a measurable form.
• The amount of the analyte present in the controls should be close to the medical decision points of the test; this means that controls should check both low values and high values.
• Controls should have the same matrix as patient samples; this usually means that the controls are serum based, but they may also be based on plasma, urine or other materials.

Because it is more efficient to have controls that last for some months, it is best to obtain control materials in large quantities.

Types and sources of control material

Control materials are available in a variety of forms. They may be frozen, freeze-dried or chemically preserved. The freeze-dried or lyophilized materials must be reconstituted, requiring great care in pipetting in order to ensure the correct concentration of the analyte.

Control materials may be purchased, obtained from a central or reference laboratory, or made in-house by pooling sera from different patients.

Purchased controls may be either assayed or unassayed. Assayed controls have a predetermined target value, established by the manufacturer. When using assayed controls, the laboratory must verify the value using its own methods. Assayed controls are more expensive to purchase than unassayed controls.

When using either unassayed or "in-house" controls, the laboratory must establish the target value of the analyte.

The use of in-house controls requires resources to perform validation and testing steps. An advantage is that the laboratory can produce very large volumes with exact specifications.

Choosing controls

Remember that QC materials are usually serum based. Universal precautions should be used when handling.

When choosing controls for a particular method, select values that cover medical decision points, such as one with a normal value, and one that is either high or low, but in the medically significant range.

Controls are usually available in "high", "normal" and "low" ranges. Shown in the graphic are normal, abnormal high and low, and critical high and low ranges. For some assays, it may be important to include controls with values near the low end of detection.

PATIENT   CONTROLS

Critical	Critical high and low range
Abnormal	
Normal	Normal range
Abnormal	Abnormal high and low ranges
Critical	
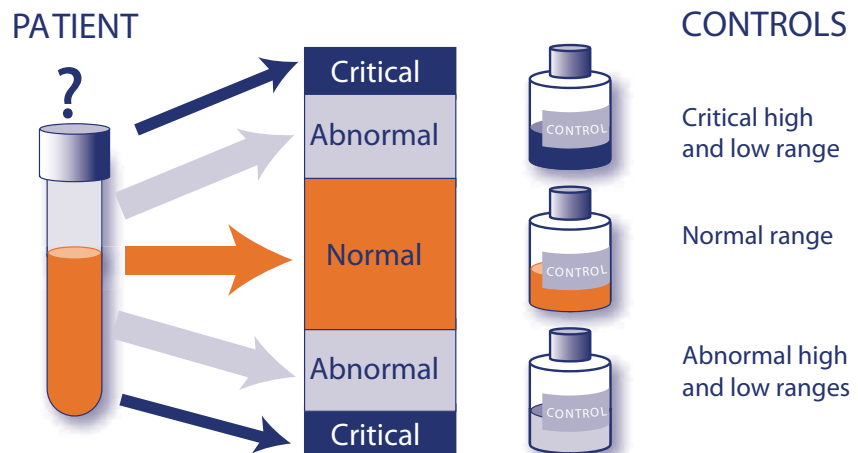
Preparing and storing control material

When preparing and storing QC materials, it is important to carefully adhere to the manufacturer's instructions for reconstituting and storage. If in-house control material is used, freeze aliquots and place in the freezer so that a small amount can be thawed and used daily. Do not thaw and refreeze control material. Monitor and maintain freezer temperatures to avoid degradation of the analyte in any frozen control material.
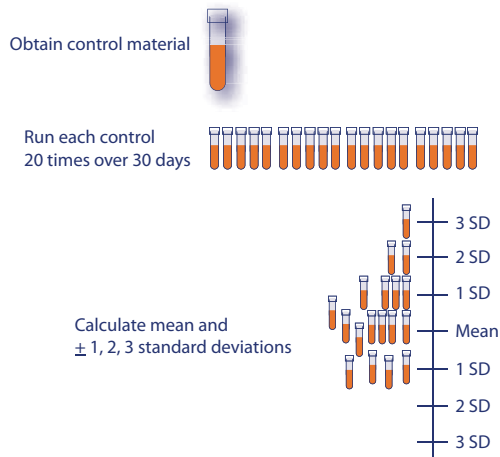
Use a pipette to deliver the exact amount of required diluent to lyophilized controls that must be reconstituted.

7-3: Establishing the value range for the control material

Once the appropriate control materials are purchased or prepared, the next step is to determine the range of acceptable values for the control material. This will be used to let the laboratory know if the test run is "in control" or if the control values are not reading properly—"out of control". This is done by assaying the control material repeatedly over time. At least 20 data points must be collected over a 20–30-day period. When collecting this data, be sure to include any procedural variation that occurs in the daily runs; for example, if different testing personnel normally do the analysis, all of them should collect part of the data.

Once the data is collected, the laboratory will need to calculate the mean and standard deviation of the results. A characteristic of repeated measurements is that there is a degree of variation. Variation may be due to operator technique, environmental conditions or the performance characteristics of an instrument. Some variation is normal, even when all of the factors listed above are controlled. The standard deviation gives a measure of the variation. This process is illustrated below.

Obtain control material

Run each control
20 times over 30 days

Calculate mean and
± 1, 2, 3 standard deviations

3 SD
2 SD
1 SD
Mean
1 SD
2 SD
3 SD

One of the goals of a QC programme is to differentiate between normal variation and errors.

A few theoretical concepts are important because they are used to establish the normal variability of the test system. QC materials are run to quantify the variability and establish a normal range, and to decrease the risk of error.

The **variability** of repeated measurements will be distributed around a central point or location. This characteristic of repeated measurements is known as **central tendency**.

The three measures of central tendency are:
- **Mode**—the number that occurs most frequently.
- **Median**—the central point of the values when they are arranged in numerical sequence.
- **Mean**—the arithmetic average of results. The mean is the most commonly used measure of central tendency used in laboratory QC.

Statistical notations

Statistical notations are symbols used in mathematical formulas to calculate statistical measures. For this chapter, the symbols that are important to know are:

Σ the sum of

N number of data points (results or observations)

X_1 individual result

$X_1 - X_n$ data point 1–n where n is the last result

$\overline{X}$ the symbol for the mean

$\sqrt{}$ the square root of the data.

Mean

The formula for the mean is:

$$\overline{X} = \frac{X_1 + X_2 + X_3 ... X_n}{N}$$

As an example of how to calculate a mean, consider enzyme-linked immunosorbent assay (ELISA) testing. The method is to gather data as ratios, add the values and divide by the number of measurements.

Before calculating QC ranges

The purpose of obtaining 20 data points by running the QC sample is to quantify normal variation and establish ranges for QC samples. Use the results of these measurements to establish QC ranges for testing.

If one or two data points appear to be too high or low for the set of data, they should not be included when calculating QC ranges. They are called **"outliers"**.
- If there are more than 2 outliers in the 20 data points, there is a problem with the data and it should not be used.
- Identify and resolve the problem and repeat the data collection.

Normal distribution

If many measurements are taken, and the results are plotted on a graph, the values form a bell-shaped curve as the results vary around the mean. This is called a **normal distribution** (also termed Gaussian distribution).

The distribution can be seen if data points are plotted on the x-axis and the frequency with which they occur on the y-axis.

The normal curve shown (right) is really a theoretical curve obtained when a large number of measurements are plotted. It is assumed that the types of measurements used for quantitative QC are normally distributed based on this theory.

Accuracy and precision
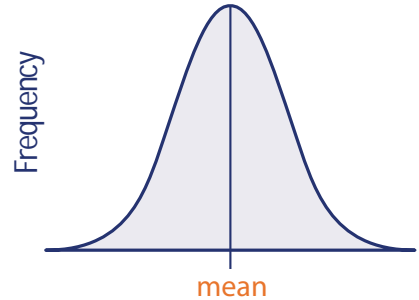
If a measurement is repeated many times, the result is a mean that is very close to the true mean.

Accuracy is the closeness of a measurement to its true value.

Precision is the amount of variation in the measurements.
• The less variation a set of measurements has, the more precise it is.
• In more precise measurements, the width of the curve is smaller because the measurements are all closer to the mean.

Bias is the difference between the expectation of a test result and an accepted reference method.
The reliability of a method is judged in terms of accuracy and precision.

Target illustration

A simple way to portray precision and accuracy is to think of a target with a bull's eye. The bull's eye represents the accepted reference value which is the true, unbiased value. If a set of data is clustered around the bull's eye, it is **accurate**.

The closer together the hits are, the more **precise** they are. If most of the hits are in the the bull's eye, as in the figure on the **left**, they are both precise and accurate.

Accurate and precise Precise but biased Imprecise
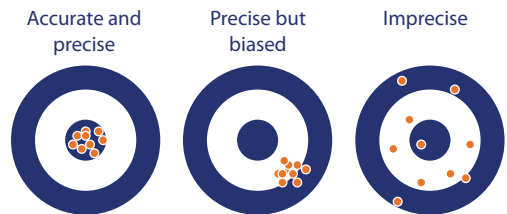
Accurate = precise but not biased

The values in the **middle figure** are precise but not accurate because they are clustered together but not at the bull's eye. The figure on the **right** shows a set of hits that are imprecise.

Measurements can be precise but not accurate if the values are close together but do not hit the bull's eye. These values are said to be **biased**. The middle figure demonstrates a set of precise but biased measurements.

Measures of variability

The purpose of quality control is to monitor the accuracy and precision of laboratory assays before releasing patient results.

The methods used in clinical laboratories may show different variations about the mean; hence, some are more precise than others. To determine the acceptable variation, the laboratory must compute the standard deviation (SD) of the 20 control values. This is important because a characteristic of the normal distribution is that, when measurements are normally distributed:
- 68.3% of the values will fall within –1 SD and +1 SD of the mean
- 95.5% fall within –2 SD and +2 SD
- 99.7% fall between –3 SD and +3 SD of the mean.

Knowing this is true for all normally shaped distributions allows the laboratory to establish ranges for QC material.

Once the mean and SD are computed for a set of measurements, a QC material that is examined along with patients' samples should fall within these ranges.

Standard deviation

SD is a measurement of variation in a set of results. It is very useful to the laboratory in analyzing QC results.

The formula for calculating standard deviation is:

$$SD = \sqrt{\frac{\sum (X_1 - \overline{X})^2}{n-1}}$$

The number of independent data points (values) in a data set are represented by "n". Calculating the mean reduces the number of independent data points to n – 1. Dividing by n – 1 reduces bias.

Calculating acceptable limits for the control

The values of the mean, as well as the values of $\pm$ 1, 2 and 3 SDs are needed to develop the chart used to plot the daily control values.

- To calculate 2 SDs, multiply the SD by 2 then add and subtract each result from the mean.
- To calculate 3 SDs, multiply the SD by 3, then add and subtract each result from the mean.

For any given data point, 68.3% of values will fall between $\pm$ 1 SD, 95.5% between

± 2 SD and 99.7% between ± 3 SD of the mean.

When only one control is used, we consider an examination run to be "in control" if a value is within 2 SD of the mean.

Coefficient of variation

The coefficient of variation (CV) is the SD expressed as a percentage of the mean.

$$CV\ (\%) = \frac{SD}{Mean}\ x\ 100$$

The CV is used to monitor precision. When a laboratory changes from one method of analysis to another, the CV is one of the elements that can be used to compare the precision of the methods. Ideally, the value of the CV should be less than 5%.

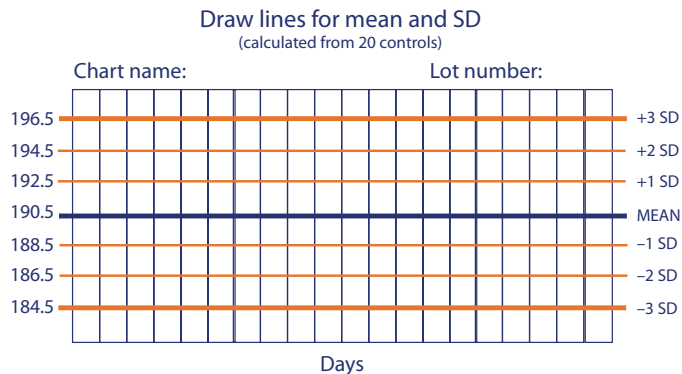## 7-4: Graphically representing control ranges

Once the appropriate range of control values has been established, the laboratory will find it very useful to represent the range graphically for the purpose of daily monitoring. The common method for this graphing is the use of Levey–Jennings charts.

In order to develop Levey–Jennings charts for daily use in the laboratory, the first step is the calculation of the mean and SD of a set of 20 control values as explained in 7-3.

A Levey–Jennings chart can then be drawn, showing the mean value as well as ± 1, 2, and 3 SD. The mean is shown by drawing a line horizontally in the middle of the graph and the SD are marked off at appropriate intervals and lines drawn horizontally on the graph, as shown below.

Draw lines for mean and SD
(calculated from 20 controls)

Chart name:		Lot number:

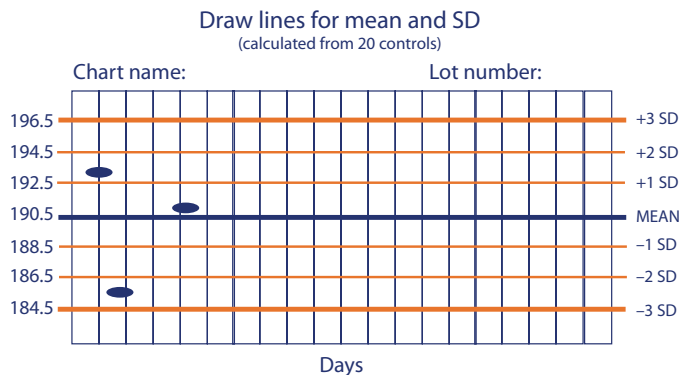|	
196.5	+3 SD
194.5	+2 SD
192.5	+1 SD
190.5	MEAN
188.5	−1 SD
186.5	−2 SD
184.5	−3 SD

Days

This Levey–Jennings chart was developed using 20 repeated measurements of the control value. In order to use the Levey–Jennings chart to record and monitor daily control values, label the x-axis with days, runs, or other intervals used to run QC. Label the chart with the name of the test and the lot number of the control being used.

7-5: Interpreting quality control data

Plotting control values

A QC sample tested along with patient's samples can now be used to determine if daily runs are "in control". A control sample must be run with each set of patient samples.

Run the control and plot it on the Levey–Jennings chart. If the value is within **±2 SD,** the run can be accepted as **"in-control"**.

Draw lines for mean and SD
(calculated from 20 controls)

Chart name: Lot number:

196.5														+3 SD
194.5														+2 SD
192.5														+1 SD
190.5														MEAN
188.5														−1 SD
186.5														−2 SD
184.5														−3 SD

Days

The values on the chart are those run on days 1, 2 and 3 after the chart was made. In this case, the second value is "out of control" because it falls outside of 2 SD.

When using only one QC sample, if the value is outside 2 SD, that run is considered "out of control" and the run must be rejected.

Number of controls used

If it is possible to use only one control, choose one with a value that lies within the normal range of the analyte being tested. When evaluating results, accept all runs where the control lies within ±2 SD. Using this system, the correct value will be rejected 4.5% of the time.

In order to improve efficiency and accuracy, a system using two or three controls for each run can be employed. Then another set of rules can be used to avoid rejecting runs that may be acceptable. These rules were applied to laboratory QC by a clinical chemist named James Westgard. This Westgard multirule system requires running two controls of different target values for each set of examinations, developing a Levey–Jennings chart for each, and applying the rules.

The use of three controls with each run gives even higher assurance of accuracy of the test run. When using three controls, choose a low, a normal and a high range value. There are also Westgard rules for a system with three controls.

Detecting error

Errors that occur in the testing process may be either random or systematic.

With random error, there will be a variation in QC results that show no pattern. This type of error generally does not reflect a failure in some part of the testing system, and is therefore not like to reccur. Random error is only a cause for rejection of the test run if it exceeds ± 2 SD.

Systematic error is not acceptable, as it indicates some failure in the system that can and should be corrected. Examples of evidence of systematic error include:
- shift—when the control is on the same side of the mean for five consecutive runs;
- trend—when the control is moving in one direction, and appears to be heading toward an out-of-control value.

Even when a control value falls within 2 SD, it can be a cause for concern. Levey–Jennings charts can help distinguish between normal variation and systematic error.

Shifts and trends

Shifts in the mean occur when an **abrupt** change is followed by **six or more** consecutive QC results that fall on one side of the mean, but typically within 95% range as if clustered around a new mean. On the sixth occasion this is called a shift and results are rejected.

Trends occur when values gradually, but continually, move in one direction over six or more analytical runs. Trends may display values across the mean, or they may occur only on one side of the mean. On the sixth occasion, this is determined to be a trend and results are rejected.

The source of the problem must be investigated and corrected before patients' samples are reported.

Measurement uncertainty

As variation occurs in measurements, uncertainty exists as to the true value. Uncertainty represents a range of values in which the true value is reasonably expected to lie. In most situations, measurement uncertainty is estimated at "95% coverage". For most instances, a range of ± 2 SD is accepted as measurement uncertainty that is explained by random variation.

But the degree of variation also depends on the method used. Methods that are more precise have less uncertainty because the amount of variation included in the 95% limits is smaller.

Laboratories should strive to use methods that have a high degree of precision, and always follow standard operating procedures.

7-6: Using quality control information

When the QC sample that is used in a test run is out of the acceptable range, the run is considered to be "out of control". When this happens, there are several steps that the laboratory must follow.

- The testing process should be stopped and the technologist must immediately try to identify and correct problems.
- Once possible sources of error have been identified and corrections have been made, the control material should be rechecked. If they read correctly, then patient samples, along with another QC specimen, should be repeated. Do not simply repeat the testing without looking for sources of error and taking corrective action.
- Patient results **must not be reported** until the problem is resolved and the controls indicate proper performance.

Problem solving

When attempting to solve QC problems, it is useful to have established policies and procedures for remedial action. Often, manufacturers of either equipment or reagents will provide guidelines that can be helpful. Use any troubleshooting guides that are available.

Possible problems to consider include:
- degradation of reagents or kits
- control material degradation
- operator error
- failure to follow manufacturer's instructions
- an outdated procedure manual
- equipment failure
- calibration error.

7-7: Summary

Summary

A QC programme for quantitative tests is essential to ensuring accuracy and reliability of laboratory testing. The laboratory must establish a QC programme that monitors all quantitative tests. The programme will have written policies and procedures that are followed by all laboratory staff.

The overall responsibility of managing the QC programme is usually assigned to the quality manager, who monitors and reviews all QC data on a regular basis. The recording of the QC data must be complete and easy to access.

For quantitative testing, statistical analysis can be used for the monitoring process, and the use of Levey–Jennings charts provides a very useful visual tool for this monitoring.

When controls are out of range, corrective action and troubleshooting must be undertaken; the problem must be fixed before reporting patient results. Therefore, good protocols for troubleshooting and corrective action are an important part of the QC process.

Key messages

- A QC programme allows the laboratory to differentiate between normal variation and error.
- The QC programme monitors the accuracy and precision of laboratory assays.
- The results of patient testing should never be released if the QC results for the test run do not meet the laboratory target values.

8. Process control—quality control for qualitative and semiquantitative procedures

8-1: Overview

Role in quality management system

Quality control (QC) is a component of process control, which is a major element of the quality management system. It monitors the processes related to the examination phase of testing and allows for detecting errors in the testing system. These errors may be due to test system failure, adverse environmental conditions or operator performance. QC gives the laboratory confidence that test results are accurate and reliable before patient results are reported.

This chapter explains how QC methods are applied to qualitative and semiquantitative laboratory examinations.


Qualitative and semiquantitative examinations

Qualitative examinations are those that measure the presence or absence of a substance, or evaluate cellular characteristics such as morphology. The results are not expressed in numerical terms, but in descriptive or qualitative terms such as "positive", "negative", "reactive", "nonreactive", "normal" or "abnormal".

Examples of qualitative examinations include microscopic examinations for cell morphology or presence of parasitic organisms, serologic procedures for presence or absence of antigens and antibodies, some microbiological procedures and some molecular techniques.

Semiquantitative examinations are similar to qualitative examinations; testing does not measure the precise
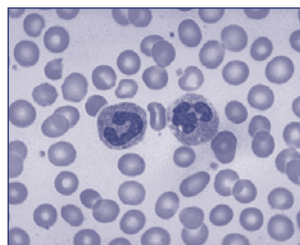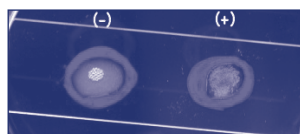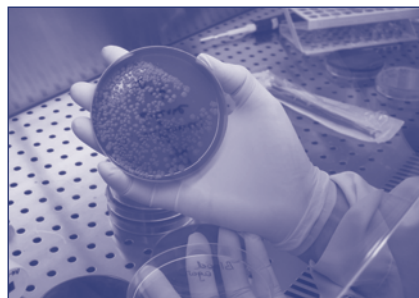
quantity of a substance. The difference is that results of these tests are expressed as an **estimate** of how much of a measured substance is present. This estimate is sometimes reported as a number. Therefore, test results for semiquantitative tests may be shown as "trace amount", "1+, 2+ or 3+", or positive at 1:160 (titre or dilution). Examples of semiquantitative examinations are urine dipsticks, tablet tests for ketones and serological agglutination procedures.

Some microscopic examinations are considered semiquantitative because results are reported as estimates of the number of cells seen per low-power field or high-power field. For example, a urine microscopic examination might report 0–5 red blood cells seen per high-power field.

Important concepts

As with quantitative procedures, it is important to verify that results of qualitative and semiquantitative examinations are correct prior to reporting them to the requesting health care provider.

Conducting QC for many of these tests is not as easily accomplished as with quantitative tests. Therefore, it becomes essential that other processes within the quality system are carefully conducted, in addition to traditional QC methods. Following are some important overarching concepts for quality that apply to qualitative and semiquantitative tests.

- Sample management is important in all laboratory testing. Examinations that are dependent on a viable organism in the sample may need closer monitoring and better communication with nonlaboratory staff (see Chapter 5).
- Dedicated, professional staff who understand the principles of QC are key to quality.
- Incubators, refrigerators, microscopes, autoclaves and other equipment must be maintained and monitored carefully (see Chapter 3).
- Positive and negative controls must be used to monitor the effectiveness of test procedures that use special stains or reagents and tests with end-points such as agglutination, colour change or other non-numeric results.
- Reagents should be stored according to the manufacturer's instructions, labelled with the date they are opened and put into use, and discarded at the expiration date (see Chapter 4).
- Keeping records of all QC processes and corrective actions is necessary for continual improvement of the laboratory quality system (see Chapter 16).
- When problems occur, investigate, correct, and repeat patient testing (see Chapter 14).

If QC results are not what are expected, do not report patient results.

8-2: Quality control materials

Control types

Qualitative and semiquantitative examinations include tests that utilize a variety of control materials. These controls may be built-in (on-board or procedural) controls, traditional controls that mimic patient samples, or stock cultures for use with microbiological examinations.

Built-in controls

Built-in controls are those that are integrated into the design of a test system such as a test kit device. Usually, the device is marked with designated areas where coloured lines, bars or dots should appear to indicate success or failure of positive and negative controls, and these controls are performed automatically with each test. The manufacturer's product instructions may also refer to these as procedural controls, on-board controls or internal controls.

Most built-in controls monitor only a portion of the examination phase, and they vary from one test to another as to what is being monitored. For example, built-in controls for some kits may indicate that all the reagents impregnated into the device are active and working properly, whereas built-in controls for other kits may only indicate that a sample was added and solutions flowed through the device correctly. It is important to carefully read the instructions provided by the manufacturer to understand what the built-in controls monitor, and to determine whether additional controls may be needed.

Examples of test kits with built-in controls are rapid tests that detect the presence of antigens or antibodies, such as those for infectious disease (human immunodeficiency virus [HIV], influenza, lyme disease, streptococcal infection, infectious mononucleosis), drugs of abuse, pregnancy or faecal occult blood.

Even though these built-in controls give some degree of confidence, they do not monitor for all conditions that could affect test results. It is advisable to periodically test traditional control materials that mimic patient samples, for added confidence in the accuracy and reliability of test results.

In some settings, these built-in controls are referred to as internal controls.

Traditional controls

Traditional control materials are made to mimic patient samples and they are tested with the patient samples to evaluate the examination component. Positive controls have known reactivity and negative controls are nonreactive for the analyte being tested. The controls should have the same composition, or matrix, as patient samples, including viscosity, turbidity and colour, in order to properly evaluate the test performance. Control materials are often lyophilized when received, and need to be carefully reconstituted before use. Some manufacturers may provide

these controls with their test kits but, more frequently, they need to be purchased separately.

Traditional controls evaluate the testing process more broadly than built-in controls. They assess the integrity of the entire test system, the suitability of the physical testing environment (temperature, humidity, level workspace), and whether the person conducting the test performs it correctly.

Positive and negative controls are recommended for many qualitative and semiquantitative tests, including some procedures that use special stains or reagents, and tests with end-points such as agglutination or colour change. These controls should generally be used with each test run. Use of controls will also help to validate a new lot number of test kits or reagents, to check on temperatures of storage and testing areas, and to evaluate the process when new testing personnel are carrying out the testing.

Things to keep in mind when using traditional controls for qualitative or semiquantitative tests are:
• test control materials in the same manner as testing patient samples;
• use a positive and negative control, preferably once each day of testing, or at least as often as recommended by the manufacturer;
• choose positive controls that are close to the cut-off value of the test, to be sure the test can detect weak positive reactions;
• for agglutination procedures, include a weak positive control as well as a negative control and a stronger positive control;
• for tests with an extraction phase, such as some rapid group A *Streptococcus* tests, choose controls that are capable of detecting errors in the extraction process.

Stock cultures

QC in microbiology requires use of live control organisms with predictable reactions to verify that stains, reagents and media are working correctly. They must be kept on hand and carefully maintained in the form of stock and working cultures. For each reaction, organisms with both positive and negative results should be tested.

The following organizations offer reference strains, which are available from local distributors:
• American Type Culture Collection (ATCC)
• National Type Culture Collection (NTCC, United Kingdom)
• Pasteur Institute Collection (CIP, France).

Purchased reference strains are usually lyophilized and kept in the refrigerator. Once they are reconstituted, plated and checked for purity, they can be used to make working cultures for quality control.

Some laboratories may choose to use isolates from their own laboratories for QC. If so, they should be monitored closely to verify that reactions tested are sustained over time.

8-3: Quality control of stains

In performing many qualitative and semiquantitative procedures, stains are needed for evaluating microscopic morphology of cells, parasites or microbes, or to determine their presence or absence. Stains are used for microscopic procedures that provide information for either preliminary or definitive diagnosis. These are frequent in haematology, urinalysis, cytology, histology, microbiology, parasitology and other laboratory areas.

In microbiology, permanent stains such as acridine orange, trichrome and iron-haematoxylin for faecal parasites, and Giemsa stain for malaria, are frequently used. Gram stains are used for identification of bacteria and yeast from colonies and samples. Acid-fast stains are particularly important for preliminary diagnosis, since growth of mycobacteria takes several weeks. In many sites, *Mycobacterium tuberculosis* (TB) cultures are not available and acid-fast smears will provide the final diagnosis for patients. For wet mounts, iodine solutions are used to detect cysts and eggs in faecal samples, and potassium hydroxide preparations are used to detect fungal elements.

Examination of blood smears requires a stain that allows for clear visualization of red blood cells, white blood cells, platelets and inclusions within cells. Differentiation of cells in blood most frequently employs a Wright stain, and some haematology procedures use special stains to help differentiate infection from leukaemia.
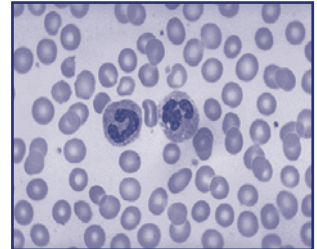
Cytology and histology tests require a wide variety of stains that provide valuable information for diagnosis. Many other stains are available to laboratory staff for special uses.

The common elements for QC are the same: the stains should be prepared and stored properly, and checked to be sure they perform as expected. Remember that many of the microscopic examinations that rely on stains are critical in diagnosis of many diseases.

Some stains can be purchased commercially, but others must be prepared by the laboratory, following an established procedure. Once stains are made, their bottles should be labelled with the following information:
• name of the stain
• concentration
• date prepared
• date placed in service
• expiration date/shelf life
• preparer's initials.

It may be useful to keep a logbook for recording information on each stain in use, including the lot number and date received. The expiration date must be noted on the label. Some stains deteriorate and lose their ability to produce the correct reactions.

Stains should be stored at the correct temperature at all times and in an appropriate staining bottle. Some stains must be protected from light. In some cases, working solutions can be made from stock solutions. If so, storage of working solutions should be carefully monitored.

Quality control

Because of their importance, stains should be checked each day of use with positive and negative QC materials, to make sure their reagents are active and they provide the intended results. In most cases, positive and negative controls should be stained with each batch of patients' slides. All QC results must be recorded each time they are run.

Stains should also be examined to look for precipitation or crystal formation, and to check for bacterial contamination. Careful maintenance and care of the stock and working solutions of stains is an essential component in a system to provide good quality in microscopic examinations.

Be aware that many stains are toxic, therefore, take appropriate safety precautions when working with them.

8-4: Quality control of microbiological media

The quality of media used in the microbiology laboratory is crucial to achieving optimal and reliable results. Some media are essential to isolation of microbes, so it is imperative that they function as expected. QC procedures provide the confidence that media has not been contaminated prior to use, and that it supports the growth of the organism with which it was inoculated.
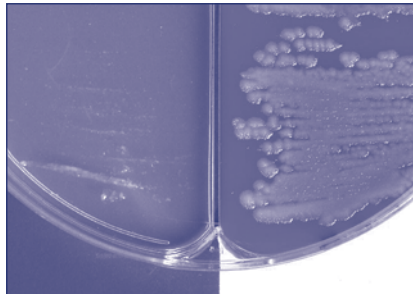
Verifying performance

The performance characteristics of all media used in the laboratory must be verified by the appropriate QC methods. For media that is prepared in-house, this evaluation must be conducted for each batch prepared; for all commercially prepared media, the performance verification will be performed for each new lot number.

In all cases, in-house and purchased media should be carefully checked for:
- sterility—incubate overnight before use
- appearance—check for turbidity, dryness, evenness of layer, abnormal colour
- pH
- ability to support growth—using stock organisms
- ability to yield the appropriate biochemical results—using stock organisms.

Use of control organisms for verification

The laboratory must maintain sufficient stock organisms to check all its media and test systems. Some examples of important stock organisms, and the media checked, include:
- *Escherichia coli* (ATCC 25922): MacConkey or eosin methylene blue (EMB), some antimicrobial susceptibility testing;
- *Staphylococcus aureus* (ATCC 25923): blood agar, mannitol salt and some antimicrobial susceptibility tests;
- *Neisseria gonorrhoeae* (ATCC 49226): chocolate agar and Thayer–Martin agar.

For selective media, inoculate a control organism that should be inhibited as well as one that should grow. Discard any batch of media that does not work as expected.

For differential media, inoculate the media with control organisms that should demonstrate the required reactions. For example, inoculate both lactose-fermenting and non-lactose-fermenting organisms onto EMB or MacConkey agar to verify that the colonies exhibit correct visual appearance.

Note: sheep and horse blood are preferred in preparing media for routine cultures. Blood agar made from human blood should not be used as it will not demonstrate the correct haemolysis pattern for identification of certain organisms, and it may contain inhibitory substances. In addition, human blood can be biohazardous.

In-house media preparation records

It is important to keep careful records for media that is prepared in the laboratory. A logbook should be maintained that records:
• date and preparer's name
• name of the medium, the lot number and manufacturer
• number of prepared plates, tubes, bottles or flasks
• assigned lot and batch numbers
• color, consistency and appearance
• number of plates used for QC
• sterility test results at 24 and 48 hours
• growth test(s)
• pH.

8-5: Summary

Examinations with non-numerical results

Qualitative and semiquantitative examinations are those that give non-numerical results. Qualitative examinations measure the presence or absence of a substance, or evaluate cellular characteristics such as morphology. Semiquantitative examinations provide an estimate of how much of the measured substance is present.

Qualitative and semiquantitative testing must be monitored by QC processes. These processes should use controls that mimic patient samples as much as possible. Quality controls that check kits, reagents, stains and microbiological media and ensure that they work as expected must be used whenever they are available.

The laboratory must establish a QC programme for all of its qualitative and semiquantitative tests. In establishing this programme, set policies, train staff and assign responsibilities, and ensure that all resources needed are available. Make sure that recording of all QC data is complete, and that appropriate review of the information is carried out by the quality manager and the laboratory director.

Key messages

- All staff must follow the QC practices and procedures.
- Always record QC results and any corrective actions that are taken.
- **If QC results are not acceptable, do not report patient results.**