

# Content Sheet 8-1: Overview of Quality Control for Qualitative and Semi-quantitative Procedures

## Role in quality management system

Quality Control (QC) is a component of process control, and 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 module explains how QC methods are applied to qualitative and semi-quantitative laboratory examinations.

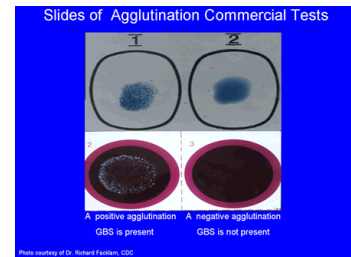


## Qualitative and semi-quantitative examinations

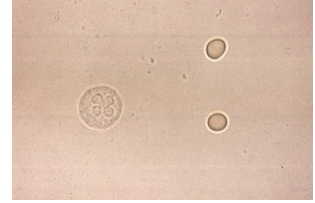
Qualitative examinations are those that measure the presence or absence of a substance, or evaluate cellular characteristics such as morphology. The results are not expressed in numerical terms, but in descriptive or qualitative terms such as “positive,” “negative,” “reactive,” “non-reactive,” “normal,”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



Semi-quantitative 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 semi-quantitative tests may be shown as “trace amount”, “1+, 2+, or 3+”, or positive at 1:160 (titer or dilution). Examples of semi-quantitative examinations are urine dipsticks, tablet tests for ketones, and serological agglutination procedures.



Some microscopic examinations are considered semi-quantitative 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 semi-quantitative examinations are correct prior to reporting them to the requesting healthcare provider.

Conducting quality control 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 quality control methods. Following are some important over-arching concepts for quality that apply to qualitative and semi-quantitative 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 non-laboratory staff (see Sample Management Module 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 Equipment Management Module 3).
- Positive and negative controls must be used to monitor the effectiveness of test procedures that use special stains or reagents, tests with endpoints such as agglutination, color change, or other non-numeric results.
- Reagents should be stored according to manufacturer's instructions, labelled with the date they are opened and put into use, and discarded at the expiration date (see Purchasing and Inventory Module 4).
- Keeping records of all quality control processes and corrective actions is necessary for continual improvement of the laboratory quality system (see Documents and Records Module 16).
- When problems occur, investigate, correct, and repeat patient testing (see Occurrence Management Module 14).



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

## Content Sheet 8-2: Quality Control Materials

### Control types

Qualitative and semi-quantitative 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 may consist of 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 colored 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 (HIV/AIDS, 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 non-reactive for the analyte being tested. The controls should have the same composition, or matrix, as patient samples, including viscosity, turbidity, and color, 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 semi-quantitative tests, including some procedures that use special stains or reagents, and tests with endpoints such as agglutination or color 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 semi-quantitative 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**

Quality control 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:

- ATCC—American Type Culture Collection;
- NTCC—National Type Culture Collection (UK);
- CIP—Pasteur Institute Collection (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.

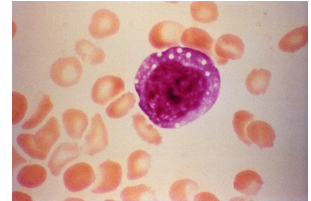
## Content Sheet 8-3: Quality Control of Stains

### Procedures using stains

In performing many qualitative and semi-quantitative 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-hematoxylin 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 KOH preparations 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.

### Stain management

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 labeled 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 log book 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 quality control 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.

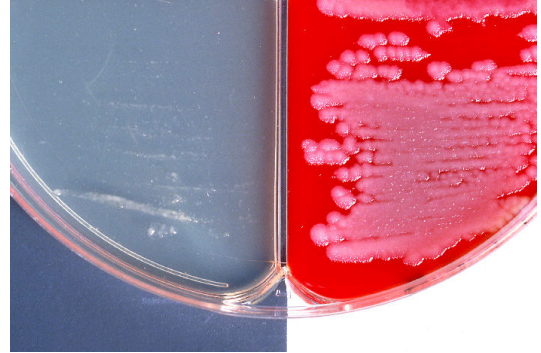


**Be aware that many stains are toxic therefore take appropriate safety precautions when working with them.**

## Content Sheet 8-4: QC of Microbiological Media

### QC is essential for 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. Quality control 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 quality control 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 color;
- 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 to 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 log book 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 batch number
- color, consistency, and appearance
- number of plates used for QC
- sterility test results at 24 and 48 hours
- growth test(s)
- pH.



## Content Sheet 8-5: Summary

### Examinations with non-numerical results

Qualitative and semi-quantitative 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. Semi-quantitative examinations provide an estimate of how much of the measured substance is present.

Qualitative and semi-quantitative testing must be monitored by quality control 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 assure that they work as expected must be used whenever they are available.

The laboratory must establish a quality control program for all of its qualitative and semi-quantitative tests. In establishing this program, set policies, train staff and assign responsibilities, and assure that all resources needed are available. Make sure that recording of all quality control 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 quality control practices and procedures.
- Always record quality control results and any corrective actions that are taken.
- **If QC results are not acceptable, do not report patient results.**