

# *Quality Control and Assessment in Flow Cytometry*

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# Quality Control in Flow Cytometry

- Internal Quality Control and QC systems
- External Quality Assessment
- Training and Competency of Staff

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The slides will focus upon the 3 main areas – Internal Quality Control and systems that should be implemented within a laboratory; how external quality assessment can be used and finally on maintaining staff training and competency to practice..

## Objectives

- ✓ What is Quality Assurance (QA)?
- ✓ How is it different from Quality Control (QC)?
- ✓ Why is it important?
- ✓ Demonstrate QA/QC for CD4 enumeration by flow cytometry.

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The objectives are clearly defined in that we will explore the difference between what is quality assurance and what is quality control. We will also explore why this is important.

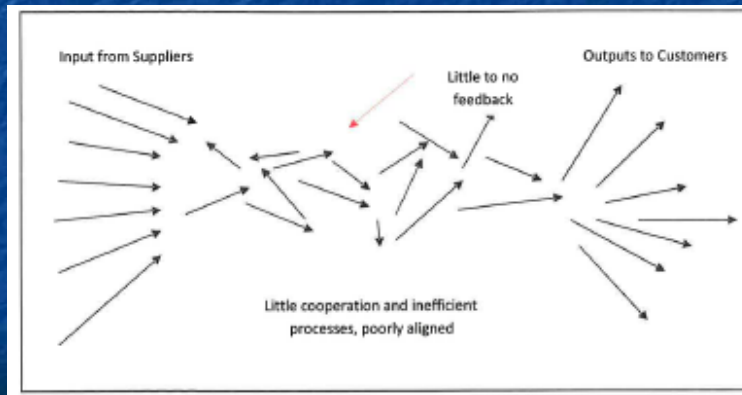
# Quality Requirements

- ✓ The customer defines Quality
  - ✓ Ordering/treating clinician
  - ✓ Patient
- ✓ Accurate and consistent test results that are standardized over time regardless of variables

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It should always be remembered that it is the customer who defines quality. This could be either the clinician making the request or treating the patient or even the patient themselves. However, the most important aspect of quality is that the results are accurate and consistent.

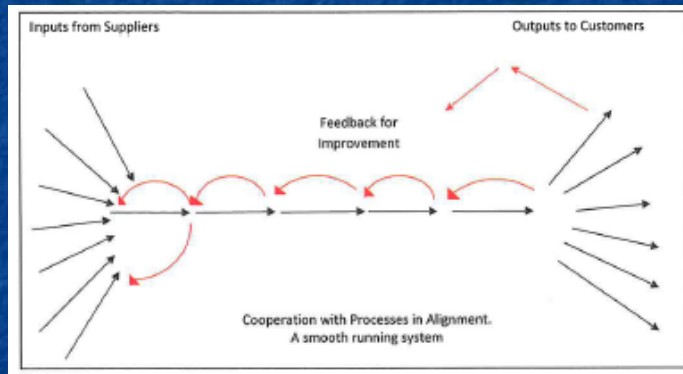
## Poorly aligned/inefficient lab process



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One of the most important principles in producing an efficient laboratory is identifying the areas where systems may be failing. In this diagram it can be seen that as requests come in to the laboratory there is no smooth work flow process causing poor communication and little interaction between areas.

## A smooth running lab system



Goal: To minimize variables (that we can control) in order to ensure identical conditions on a daily basis.

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However, once the laboratory is subjected to review (this may be due to introducing accreditation or LEAN management processes) then increased cooperation occurs leading to better communication and ultimately efficient processes. This in turn will increase output and ensure that the data generated is accurate and timely.

# Variables

- ✓ Sample handling/integrity
  - ✓ Handling at collection
  - ✓ Anticoagulant, time, temperature
- ✓ Staff
  - ✓ Differences in techniques
  - ✓ Ability to recognize problems
  - ✓ Adherence to SOP

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However, to achieve this efficient workflow we have to examine what the variables are that may impact on our processes. The two main areas that impact on our processes are the sample and staff. With respect to the sample the results may vary on how, when and where the sample was obtained – for example some tests, such as in neutrophil testing, require samples to be less than 6 hours old whereas other tests such as CD4 T lymphocyte counting can be performed within 24 hours. Therefore it is important that transportation systems are in place that can meet these requirements. Other factors to consider are type of anticoagulant and storage/transportation temperature. With respect to anticoagulants it is well known that heparin based anticoagulants can affect morphology but may be better suited to tests involving platelets. A critical requirement is the storage and transportation temperature. Clearly too high a storage temperature will increase the degradation rate rendering the sample unsuitable for analysis, however, specimen transportation fluids are available that may help. Generally, storage of specimens is best at 15°C - 25°C. Specimens can be stored in a refrigerator but systems must be in place to monitor the temperature of the refrigerator and the specimen must be allowed to warm to room temperature with constant mixing before use. It should be remembered that the most important asset of the laboratory are the staff. The staff should be trained appropriately and be able to recognise problems and how to resolve them. They should also ensure that they adhere strictly to the laboratory SOPs.

# Variables

- ✓ Equipment
  - ✓ Different outputs day to day
  - ✓ Multiple Instruments
  - ✓ Multiple Laboratories
- ✓ Reagents
  - ✓ Different Lots of Reagents
  - ✓ Change in fluorescence/output
  - ✓ Contamination

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Other areas that impact upon the quality of the laboratory results are both the instrument and reagents used. The equipment should be subjected to rigorous daily monitoring with the performance of the instruments record daily. Multiple instruments should be cross calibrated to ensure comparable performance. In addition, the reagent performance should also be monitored and recorded. This requires the when lots are changed and that continuity data has been recorded such that if there are any differences in the new batch of reagent performance these can be identified. One important consideration when using reagents is how cross contamination can impact upon the results. This may be contaminating an antibody with another antibody that will affect the staining profiles or allowing bacterial contamination of a reagent such as sheath fluid which will then be seen as increased events on the flow cytometer.



## Why Standardize?

- ✓Meet customer requirements
- ✓Consistency and reliability of results
  - ✓Monitoring CD4 over time
  - ✓Results impact patient care
- ✓Credibility in the field and as a technology
- ✓Reduce waste and rework (thereby cost)

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So why do we standardise? The reasons are several fold but as mentioned earlier this is to ensure that the data output meets the customers expectations and ensure consistency and reliability of results over time. It should be noted that failure to ensure a quality output will impact upon the patient for example they could receive the wrong or delayed treatment. An example of monitoring the results is by introducing consistency checks, for example, ensure that if there are two antigens that are detected by the same antibody being tested within a panel then the results match. All these procedures will increase the credibility of the laboratory in the field and make the laboratory more efficient by reducing wastes and costs.



A "**standard**" is—something that is done the same way every time with similar/same results.

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Ultimately a standard is ensuring that everything is done exactly the same way every time such that the output is consistent.

## Quality Assurance (QA)

Planned and systematic set of activities to provide adequate confidence that requirements for quality will be met and correct results are reported.

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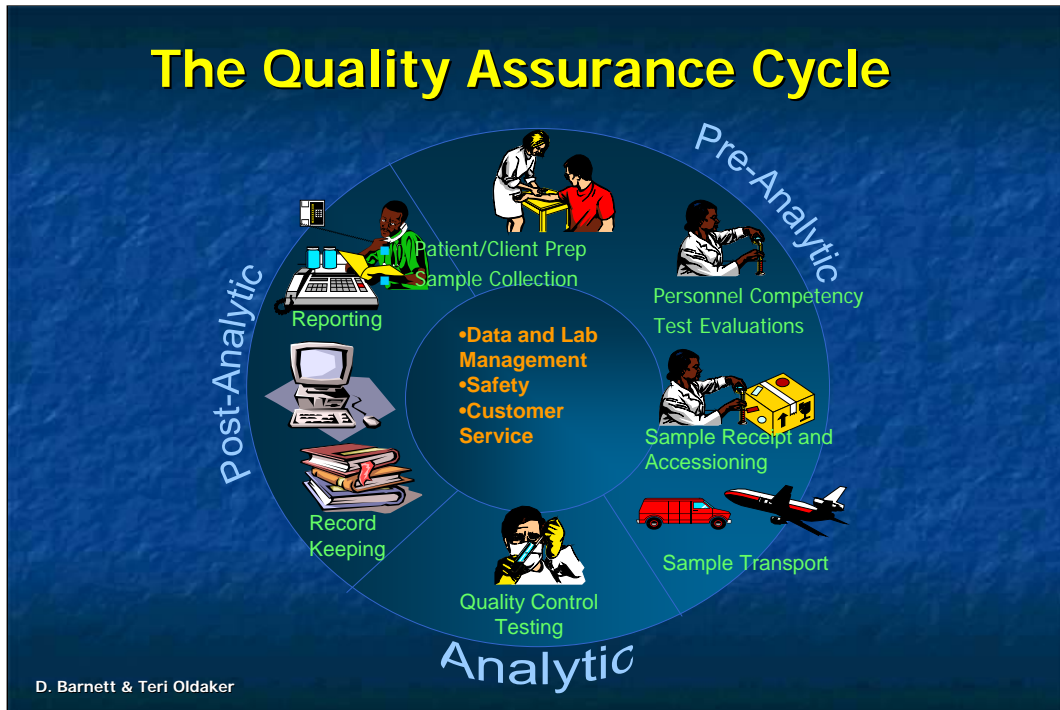
So how do we ensure quality? To achieve this we have to plan a systematic process that will ensure that all our objectives are met and that they conform to the standards that have been set out.

## Quality Assurance (QA)

- ✓ *The right result,*
- ✓ *Right time,*
- ✓ *Right specimen,*
- ✓ *Right patient,*
- ✓ *Correct reference data*
- ✓ *Right price*
- ✓ at the
- ✓ on the
- ✓ from the
- ✓ with result interpretation based on
- ✓ and at the

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The above slide sets out all the principles of quality assurance.



The quality assurance cycle can be split into 3 areas: Pre-analytical, analytical and post analytical. We have covered the pre analytical and analytical stages of this cycle earlier but the post analytical stage introduces areas so far not discussed. These include the record keeping and data reporting. Systematic checks should be introduced that the laboratory can return to any results that may be subsequently be found to be affected by one of the earlier parts of the cycle.

## Components of Quality Assurance

- ✓ Quality Control
- ✓ Proficiency testing (External Quality Assessment)
- ✓ Staff training/competency
- ✓ Policies/procedures/records
- ✓ Inspections/Audits
- ✓ Quality Improvement

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Thus, the components of the quality assurance cycle include quality control – a daily record of instrument/reagent and staff performance; proficiency testing – ensuring that the laboratory is participating regularly in external proficiency exercises that allows direct comparison with the laboratories peers; Staff training and competency – it is vitally important that all staff are appropriately trained and participating in continued professional development programmes; The laboratory should have well documented laboratory policies, procedures and records that detail every aspect of running a laboratory and are available for instant recall should a problem arise; Inspections and audits are an important part of the laboratory's working quality cycle. The external inspections should ideally be undertaken on a yearly basis but a planned and systematic approach to audits should be well documented with any findings recorded and any outcome/action recorded and followed up. If all these processes are followed then this should improve the quality of the laboratory's work.

## Quality Control

A set of procedures performed by the laboratory staff for the **continuous** and **immediate** monitoring of laboratory work in order to decide whether the results are reliable enough to be released.

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So what is quality control and how does this differ from quality assurance? The slide above summarises this most effectively.

# Quality Control

- ✓ Operational techniques and activities used to fulfil requirements for quality, for example those outlined by International Organisation for Standardisation (ISO).
- ✓ Internal quality control (IQC) – set of procedures for continuously assessing laboratory work and the emergent results; immediate effect, and should actually control release of results.

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The quality control (not quality assurance) of a laboratory actually falls into two key areas: Operational techniques that will be determined by the laboratory and should fulfil the standards laid out for achieving ISO (or equivalent) standards. These activities are underpinned by internal quality control procedures (IQC) which should be in place to enable the continuous assessment of laboratory results and will ultimately govern the release of such results ensuring their accuracy.



# Internal Quality Control

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## Flow Cytometry QC

- ✓ Instrument QC
- ✓ Antibody QC
- ✓ Reagent QC
- ✓ Procedural/method control
- ✓ Specimen integrity verifications
- ✓ Patient (internal) QC

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Now we will focus upon specific areas that need to be considered when undertaking flow cytometry quality control. In essence this actually covers all the areas we have just been discussing generally but with some slight amendments/additions.

## Instrument QC (Set-up and Monitoring)

- ✓ Manufacturers optimize the following:
  - ✓ Optical alignment
  - ✓ Electronic standardisation
  - ✓ Sensitivity/linearity
  - ✓ Compensation
  - ✓ Multiple instrument correlation
- ✓ Laboratory monitors and verifies daily that flow cytometer performance is consistent with established specifications

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Flow cytometers used in clinical arena are generally “black box” technology with most of the instruments setup being established during manufacturer or delivery. However, because the instrument is a highly sensitive piece of laboratory equipment a routine sequence of quality control procedures need to be established first that will enable the end user to effectively monitor the instrument performance. The optical alignment generally does not need to be adjusted by the user and is best undertaken by a trained engineer. However, electronic standardisation, sensitivity and linearity, compensation and cross instrument calibration needs to be undertaken on daily. This will ensure that the instrument is performing to the expected and predefined standards that have been established within the laboratory. Optical alignment, sensitivity and linearity should be performed once per week on the same day and at the same time with compensation undertaken daily. However, all the parameters should be checked and re-established if there has been any maintenance work undertaken – and preferably in the presence of the engineer – there is nothing worse than letting the engineer go, checking the instrument only to find that something drastic has changed and they need to return.

# Internal Quality Control

- **Standards**
  - Alignment Beads, Reference Beads, Compensation Beads
- **Verification of Instrument Performance**
  - Levy-Jennings Type Plots, Trends, Time
- **Biological Controls**

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So how can we check these parameters? Generally there are three main tools that we have at our disposal: Standards which consist of alignment beads (generally used for cell sorters), reference beads (these have a predefined level of fluorescence and should consistently fall within a given channel range – predefined by the user) and of course compensation beads (note compensation beads are used to give a “ball park” setting which should be finely tuned using biological specimens). All the parameters that are collected should be plotted on Levy-Jennings type plots as this will aid the user in being able to spot trends over time that may indicate something may be about to go wrong with the instrument. Finally, we should use biological controls as these will enable the user to determine if the instrument is behaving in the manner in which it is expected and also act as a full process control (FPC). The FPC facilitates the user to actually identify areas with specimen staining etc. that fall outside instrument QC but will impact upon the results obtained.

## Internal Quality Control Standards

### ■ Bead Classification:

- Type 0 (*Certified Blank*)
- Type I (a & b) (*Alignment*)
- Type II (a, b & c) (*Reference*)
- Type III (a, b & c) (*Calibration/Antibody Binding*)

For a more detailed explanation see Schwartz *et al*,  
Cytometry (1998) 33:106-114

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Generally the use of beads for instrument performance monitoring fall into 4 main categories. These enable the user to establish Alignment (if required) (Type I) but also help establish what the base line level of fluorescence is (certified blank) (Type 0) and also reference beads with predefined fluorescent characteristics (Type II). Generally, Type III beads are used to establish antibody binding capacity. A full explanation of the beads and how they are used can be found in the highlighted reference.

# Verification of Instrument Performance

- **System Verification**

- **Check Alignment**

- % CV, Mean Channel, PMT Voltages (for all channels) daily or after lot change

- **Check Reference**

- % CV, Mean Channel, PMT Voltages (for all channels) daily or after lot change

- **Check Compensation**

- Record daily for either 2-, 3- or 4+ system

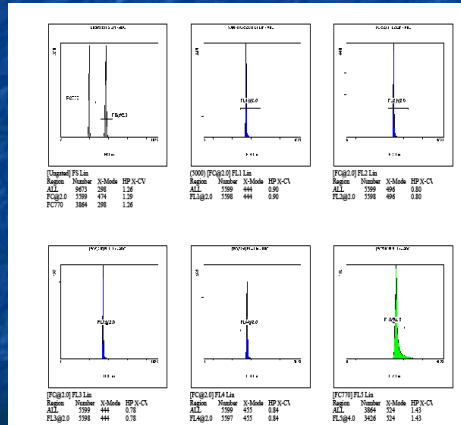
- **Time as a Parameter**

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Selective use of the beads highlighted in the previous slide will enable the user to monitor a variety of parameters, all listed here. The values that are generated should be plotted on a Levy-Jennings type plot and monitored frequently. One tool however, that can be used in real time is Time. Many instruments allow the plotting of time versus fluorescence or time versus counts which will highlight instrument performance whilst the sample is running. This will be covered shortly.

# Optical Alignment

- ✓ Alignment performed by the manufacturer at setup after major service
- ✓ Target ranges established (FL and LS)
- ✓ Verified/documented daily by lab



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Here are a few examples of how alignment can be monitored and shows the target values into which the beads should fall. All IQC should be performed before any clinical samples have been run.

## Time as a Parameter

- Generate histograms of time vs. count and time vs. fluorescence
- Since these are individual histograms no Levy-Jennings plots can be generated but it is important to compare histograms on successive days.

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The following two slides indicate how time can be used as a parameter as detailed earlier.



# Time as a Parameter

- **Alignment**
  - Plot % CV vs. Time for all parameters
- **Reference**
  - Plot Mean Channel vs. Time
- **Compensation**
  - Plot % Compensation vs. Time
- **Calibration**
  - Dependent on standard used e.g. MESF, ABC vs. Time

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It should be stressed here that all this analysis is undertaken in real time and that fluctuations occurring within the analysis period (usually around 120 seconds) should be taken as an indication that something is amiss in the system. For example Fluorescence vs. Time fluctuations should alert the user to the fact that there may be anomalies within the optical system, particularly the laser output. Whereas time versus reference (count) fluctuations would indicate a problem within the fluidics.

# Interpretation & Troubleshooting

## ■ Trends

- Unacceptable Criteria, i.e. establish tolerance
- Values outside Established Ranges

## ■ Detection Methods

- Levy-Jennings Analysis
- Analysis of Histograms when using Time
- Review of Patient data & consistency checks

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For all internal quality control data we are trying to spot trends and anything outside the pre-established tolerance levels should indicate something needs to be done. Quite often using such criteria problems can be spotted that if left will become major problems, such as laser breakdown. Using such information means that we can order and replace a laser with minimal disruption rather than waiting for the laser to completely blow and then have significant down time.

# Interpretation & Troubleshooting

- **Identification & Prevention of Instrument Error**
  - Implement Stringent QC Protocols that Promote Early Detection
  - Regular Instrument Maintenance
  - Consistency Checks for Each Sample from Each Patient
  - Specific Panels to include Consistency Checks

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Thus, the key to implementing good IQC is to promote early detection that can minimise down time. This can be achieved through regular instrument maintenance, monitoring consistency checks for each sample. Panels should therefore be designed to include such consistency checks which will ultimately improve patient results.

## Instrument QC frequency

- ✓ Verify laser output - **Daily**
- ✓ Verify optical alignment - **Daily**
- ✓ Monitor analysis ranges - **Daily**
  - ✓ Light scatter
  - ✓ Fluorescence
- ✓ Verify compensation - **Daily**
- ✓ Verify sensitivity and linearity – **Monthly\*\***
- ✓ Verify multiple instrumentation so they give the same results – **Bi-annually**

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Here is a summary of those IQC checks that should be made and the frequency with which they should be undertaken. Sensitivity and linearity need only be taken monthly if other checks suggest there may have been an issue during that period or there has been a service/repair.

## Reagent QC (Antibody)

- ✓ **Purpose:** Verify/validate the antibody reactivity is consistent with 1. lot to lot and 2. with each use. (see procedural QC)
- ✓ **Materials:** 1. Patient samples, cultured cells (for leukaemia work as commercial controls are currently unavailable) 2. commercial controls (for CD4 & CD34 work).
- ✓ **Goal/target:** Objective criteria that demonstrates equivalence. Within inter-assay CV, % positive, abs count, MFI.

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The next couple of slides discuss the IQC of reagents. This is fairly complex area but it should ensure that we are able to identify lot-to-lot variations and when a reagent is not performing at its optimum. Again, as with the instrument set up QC we should have pre-defined criteria that will enable us to demonstrate that any new reagent is behaving as we would expect and in comparison to the reagent just finished. Such criteria used could be either determining inter-assay CV and then ascertaining if the new reagent falls within expected performance criteria or use known positive cases to ascertain if the new reagent gives comparable results to those obtained with the old batch.

## Reagent QC (non-antibody reagents)

- ✓ **Purpose:** Validate each new lot of reagents is equivalent to prior lot before putting in use (and, IMPORTANTLY, before the reagent runs out!).
- ✓ **Materials:** Concurrent run with old and new lot of reagent.
- ✓ **Goal/target:** Objective criteria that demonstrates equivalence.

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The most important message to take away here is that the reagent validation should be undertaken **BEFORE** any of the existing reagents expire or run out so that at equivalence can be demonstrated.

## Biological Controls

- Immunophenotyping is a very complex process and therefore should be monitored with representative controls
- Do not use a 'fresh' daily specimen to provide IQC
  - biological and diurnal variation
  - optimization only
- Use a commercially available Whole Blood Control

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In recent years the use of stabilisation techniques has made biological controls readily available which allows the full process of sample preparation, acquisition and analysis to be monitored. The commercially available biological controls come with pre-defined target values that may be instrument/platform specific. However, the laboratory should verify the ranges prior to use. It should be stressed that a fresh daily specimen should never be used to provide IQC as there will be diurnal variation and specimen degradation that will not allow day-to-day consistency checks. A fresh specimen should only be used for instrument optimisation such as compensation following bead set-up.

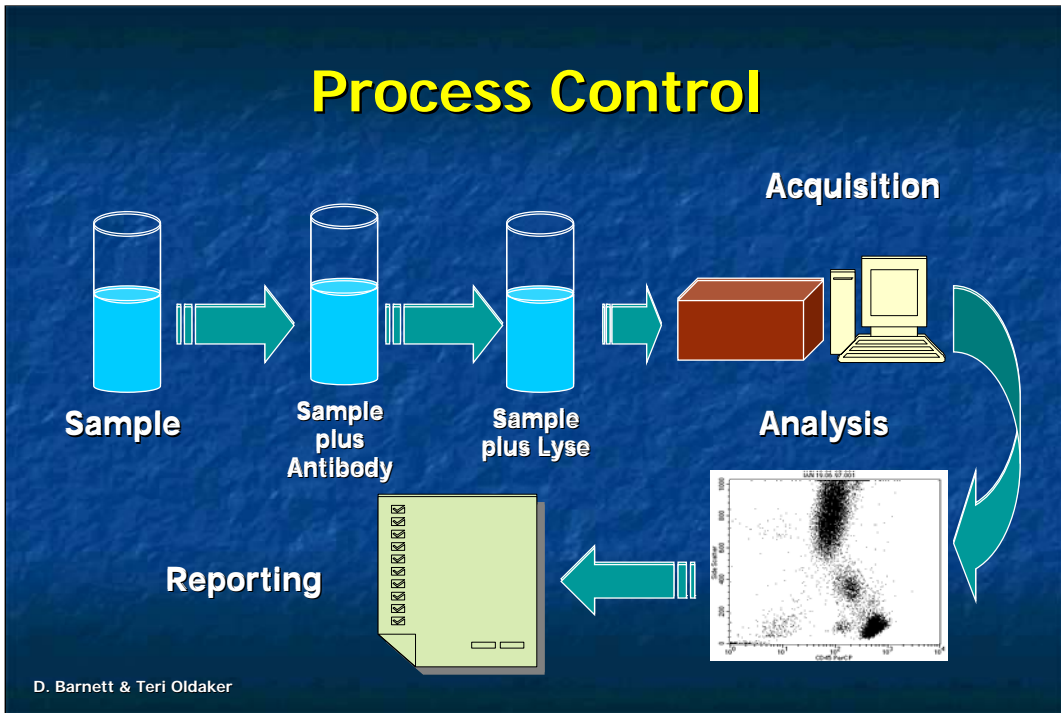
## Procedure/Method QC

- ✓ **Purpose:** Detects problems with sample preparation. Can assess compensation and serve as daily antibody control.
- ✓ **Materials:** Commercial control (2 levels), and/or healthy patient sample. It should be noted that in leukaemia flow cytometry commercial controls do not exist and therefore known patients samples should be used
- ✓ **Goal/target:** Each analyte within the laboratories established  $\pm 2$  SD range

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It is best to use a multi-level biological control that mimics the range of values encountered in clinical practice. For example if the majority of patients encountered are HIV+ patients that have low CD4 counts then the biological control should have CD4 T lymphocyte levels that are both low and in a higher range.





This slide shows the various stages that a process control should monitor from Sample Acquisition/preparation through to data reporting.

## Commercial Biological Controls

- Many commercially available controls available
  - **Part Process** - Cytotrol (Beckman-Coulter) & CD-Chex (Streck Labs. Ltd)
  - **Full Process**
    - Status Flow (R&D Systems)
    - BD MultiCheck Controls
      - CD4, CD34 & LeucoCount
    - CD-Chex Plus (Streck Labs. Ltd)
    - Fluorotrol (BioErgonomics Inc.)

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Many commercially available controls are now available some are part process controls whilst others are full process controls. A list of some of those (not exhaustive) is provided.

## Procedure/Method QC

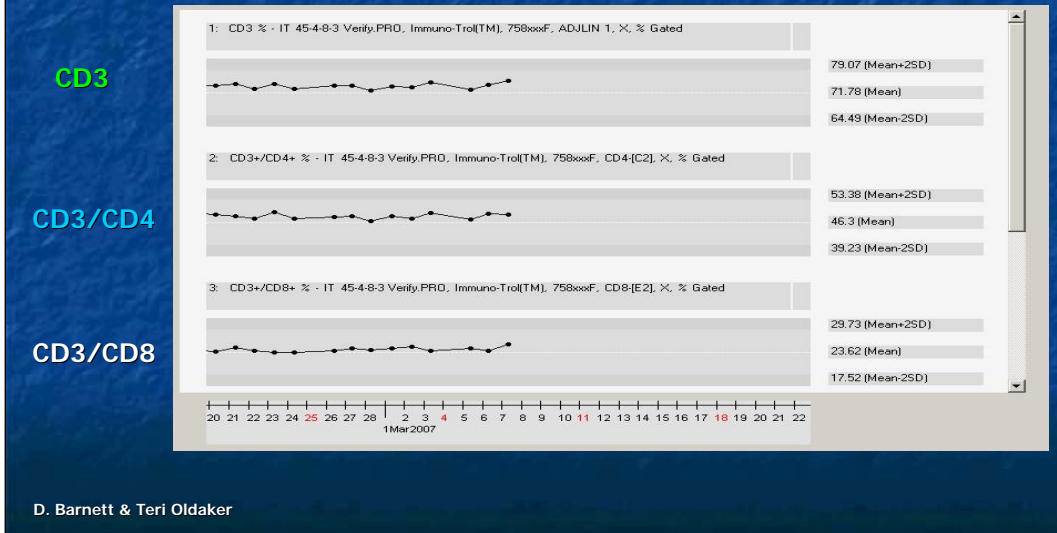
### CD4 enumeration

- ✓ Run with each staining batch of samples
- ✓ Two levels of control (normal and clinical decision point)
  - ✓ Establish your laboratory ranges (+/- 2SD)
  - ✓ Manufacturers ranges can vary up to 25%
  - ✓ CVs should be aligned with your inter assay CV
  - ✓ Do not report patient results if controls are out of range
  - ✓ Document corrective action
- ✓ Display antibody QC longitudinally on Levy-Jennings plots to detect shifts and trends.

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Once the process control of choice has been determined the control should ideally be run with each batch of specimens and, as previously mentioned be of at least two different levels that match the clinical range expected. It should be noted that manufacturers ranges can vary by as much as 25% and thus the true working range should be established before use. One useful aspect of using biological controls is that their variance should match those expected by the individual staff (or specimen processor) undertaking the testing. It is important to stress that clinical samples should not be run if the biological control values do not fall within the expected range. The samples should be re-run and all corrective action should be documented. The results, as mentioned previously, should be plotted on a Levy-Jennings plot.

# Longitudinal Display (Levy Jennings plot)



Here we can see how a biological control has been tested daily and plotted on a Levy-Jennings plot

# Specimen Integrity

- ✓ Visual inspection
  - ✓ Hemolysis or clotted – reject
  - ✓ Partial draw – depends on anticoagulant
  - ✓ Improper labeling- reject
  - ✓ Temperature extremes/lipaemia – note for consideration
- ✓ Verify cell count is optimized (<20-30x10<sup>9</sup>/L)
- ✓ Viability

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Of course, before processing any clinical specimen we should be undertaking a rigorous inspection of the sample. This will include checking for haemolysis, the age of the specimen, whether there is haemodilution by liquid anticoagulant due to an under filled specimen tube or if there are blood clots present. These will all impact on the quality of the specimen and especially the viability. If in doubt reject. However, one important point is to ensure that the specimen is labelled correctly and matches with the details provided on the request profile. Each department will, or should have, their own guidance documents in place on what are acceptability criteria.

## Internal QC (Patient)

- ✓ Single Platform Technology: Time should be constant when acquiring multiple tubes on same patient
- ✓ Delta checks: review immediately prior analysis
- ✓ Correlate with other laboratory tests (i.e. viral loads)

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Another important part of IQC is the review of patient data. Delta checks should be constantly performed on the whole range of patient data as this will act as a flag if values are outside those normally expected. When undertaking flow cytometry using tests such as single platform then the time taken to acquire should be constant between specimens. Any deviation from the expected time may indicate a problem and this should be investigated and if so the samples re-run. Finally, it is important to not take the results in isolation. If results are unexpected (but consistent when repeated) then a final check may be to correlate with other laboratory tests. For example, if a CD4 count is unexpectedly high (or low) it may be worth checking the patients viral load or treatment regimen.

## Instrument Maintenance

- ✓ Flow cytometers
- ✓ Refrigerators/Freezers
- ✓ Pipettes (calibration)
- ✓ Incubators
- ✓ Hoods

*Minimum requirement: Follow and document all manufacturers maintenance visits*

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An area that often gets overlooked are the equipment maintenance logs. All equipment should be regularly maintained and action detailed and logged accordingly. Any changes to why the instrument maintenance schedule was not in keeping with the manufacturers schedule should be justified and logged.

# Instrument Maintenance

## Document:

- ✓ The selection and acquisition
- ✓ Installation/initial calibration
- ✓ Routine maintenance
- ✓ Service and repair records
- ✓ Troubleshooting
- ✓ Disposition of old equipment

*Retain records in lab for life of equipment or minimum 2 years*

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In addition to the maintenance schedule being adhered to accreditation standards require that any acquisition and disposal of instruments are recorded along with the maintenance and repair records. These should be kept for a minimum of 2 years or for however long local regulations dictate.



## External Quality Assessment (EQA) (Proficiency Testing)

EQA is a system in which the performance of a laboratory is assessed **periodically** and **retrospectively** by an independent outside agency to indicate to the laboratory staff where there may be shortcomings and hence a need to improve/change QC procedures.

In flow cytometry, this should be undertake a **Minimum of 3 times per year** for each area under investigation.

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At this point it is useful to introduce the concept of external quality assessment (EQA) or proficiency testing (PT). EQA should always be undertaken because it is a useful adjunct to IQC in that it can provide the end user with extremely valuable information regarding methods, education and peer comparison. A laboratory should participate in EQA (depending on analyte) a least 3 times per annum. Indeed, for many countries EQA/PT testing is mandatory. The participant should participate in EQA/PT programmes that have been accredited (either by a professional body) or to ISO17043 standards. EQA/PT programmes **MUST** be independent of manufacturer bias and not affiliated to any product or reagent.

# External Quality Assurance

- Many EQA schemes now exist in flow cytometry. A short list of a few are given below (this is not exhaustive but these are the ones that operate internationally (at least 2 countries involved) – many national and local schemes also operate but are not provided here):
  - **For CD4<sup>+</sup> T lymphocytes**
    - QASI Program
    - The CAP Program
    - South Africa Program (NHLS SA)
    - UK NEQAS for Leucocyte Immunophenotyping
  - **For Leukaemia's**
    - UK NEQAS for Leucocyte Immunophenotyping
  - **For CD34<sup>+</sup> stem cells**
    - UK NEQAS for Leucocyte Immunophenotyping
  - **For PNH Testing**
    - UK NEQAS for Leucocyte Immunophenotyping

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This provides a list of EQA/PT programmes that are known to distribute material internationally with one of the largest EQA programmes in operation in flow cytometry being UK NEQAS for Leucocyte Immunophenotyping that currently provides 15 EQA programmes in molecular and flow cytometry. Further details can be obtained directly from the organiser or visiting their website, for example [www.ukneqasli.org](http://www.ukneqasli.org)

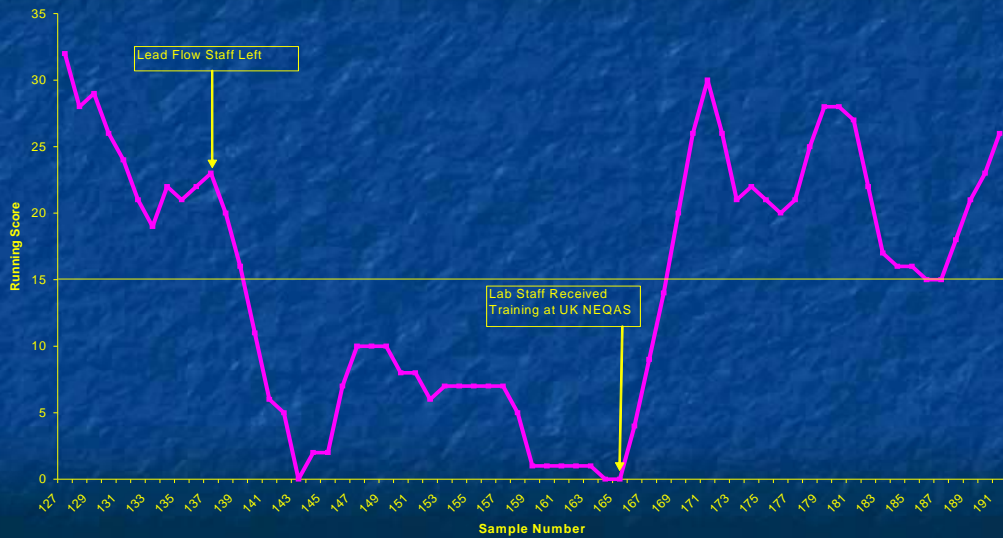
# External Quality Assessment

- Because of large numbers of participants robust data can be generated such as:
  - Facilitates direct inter-site comparison
  - Facilitates performance assessment
  - Facilitates detailed methodological analysis
  - Increasingly required for regulation
  - Primarily educational
  - Can influence general practice

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One misunderstanding of EQA is that it is often referred to as Quality Assurance – this is actually incorrect as EQA does not confer assurance (this is only conferred after all relevant IQC, EQA training and etc.) are taken into account. EQA actually provides quality ASSESSMENT. Thereby assessing the competency of a laboratory at a given point in time on a regular (usually 1-2 month interval) can only provide an indication if there are faulty processes at a given time point on a given day. Any EQA failings should always be reviewed alongside the IQC data. However, an important ethos of EQA is that it must be educational in nature but importantly it can affect clinical practice.

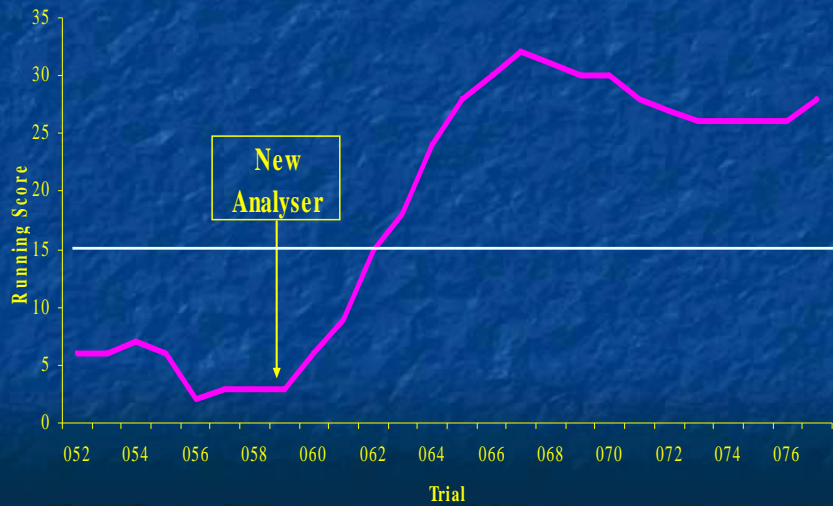
## Effect of Key Staff Departure and UK NEQAS Remedial Action



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The following 4 slides show the impact that EQA can have on laboratory practice. This shows how the departure of key staff and how retraining influences laboratory performance. The horizontal line at 15 points indicates the minimum level of acceptable laboratory performance where a laboratory was deemed to be performing unsatisfactorily until the staff were retrained and then performance improved (indicated by their running performance score between send outs being above 15 points)

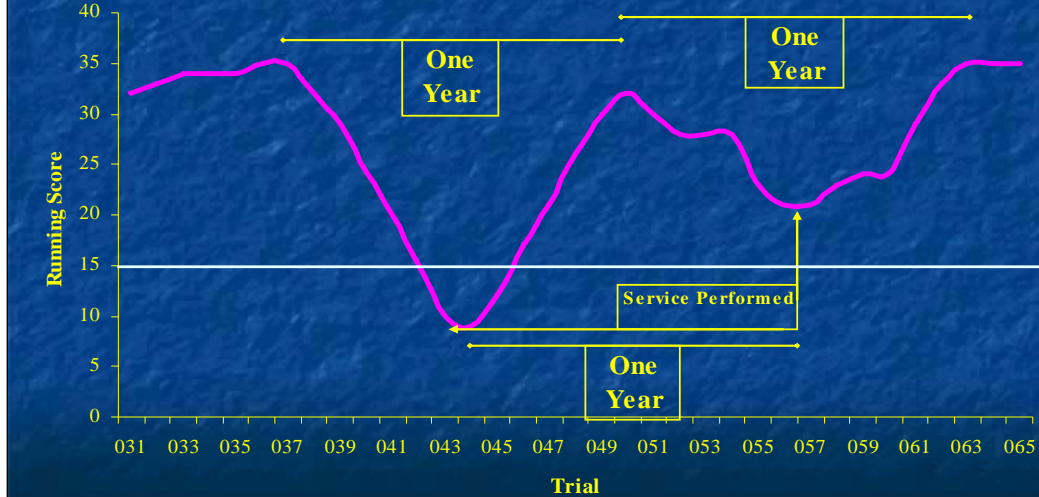
## Effect of Changing From 2-colour to 3 Colour



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Here we can see how the effect of changing technologies and approaches has helped improve a laboratory from continually being below the satisfactory line (<15 points) to achieving satisfactory status.

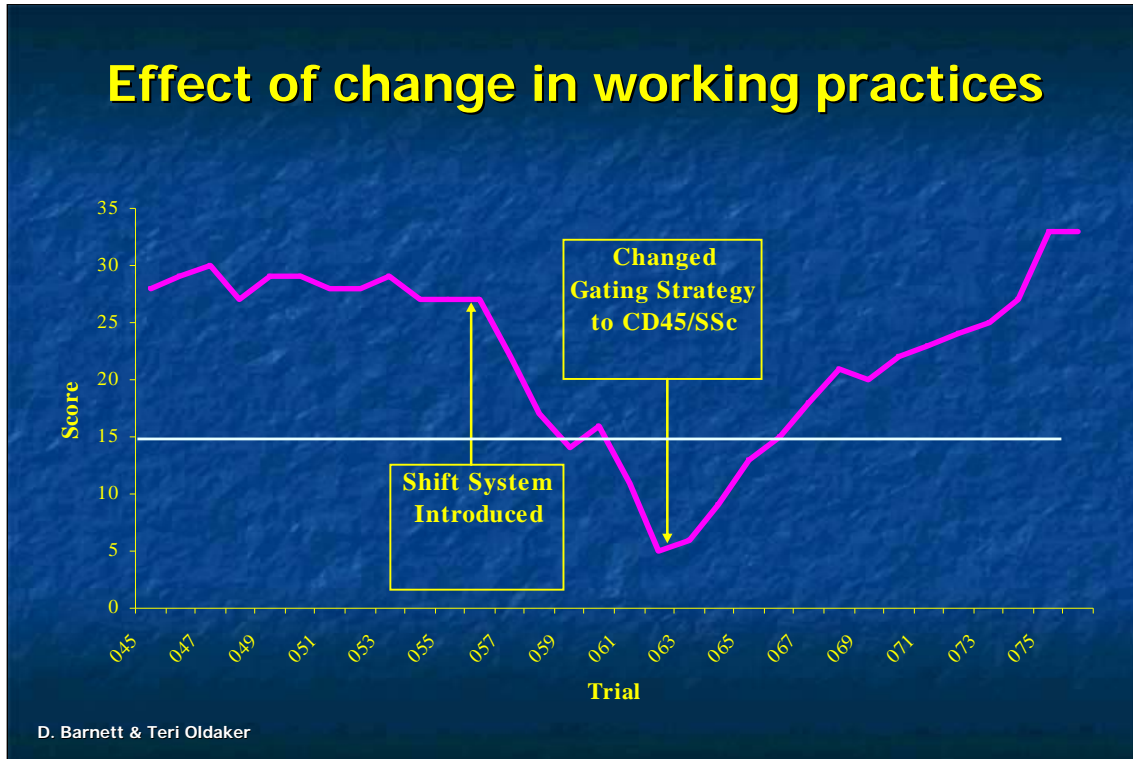
## Effect of Instrument Service on Performance



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This slide is one of the most striking as it shows how laboratory performance was cyclical and no IQC or instrument monitoring was undertaken such that between instrument service they became unsatisfactory in performance. However, when the laboratory introduced IQC (which included regular instrument monitoring) their performance improved and was maintained.

## Effect of change in working practices



This slide illustrates quite clearly how changes in laboratory practice can affect laboratory performance. In this instance two issues were occurring: First the laboratory changed to a shift system to save money but the key individual who was responsible for performing CD4 T lymphocyte counts was taken out of the laboratory to work the shift system leaving inexperienced personnel to undertake the CD4 counting however, the second issue was that they were using a Forward Scatter (FSC) versus Side Scatter (SSC) gating strategy but changed to a more efficient CD45 Versus SSC gating strategy thereby improving performance.

# How can we achieve Standardisation?

- As standardisation underpins the whole mechanism of accuracy how can this be achieved?
  - Teaching and Training
  - Good Laboratory Practice
  - Good Internal Quality Control
  - External Quality Control
  - Appropriate Instrument Validation
  - National/International Guidelines
  - International Reference Preparation

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EQA and the results it generates can have a wide impact on laboratory practice and helps to achieve standardisation. Not only can EQA influence laboratory practice it can help in delivering and achieving standardisation. Standardisation is multifaceted but takes into account all the issues we have discussed plus helps to generate national and international guidance documents and international reference standards. Without standardisation the results cannot be compared across sites either locally, nationally or internationally thus having major impact on clinical drug trials and patient well being.



## Training

“Initial” staff training – Providing adequate skills (theory and hands on experience) prior to testing and reporting of patient results.

- ✓ Formal Medical technology coursework
- ✓ Manufacturers instrument training
- ✓ Standardized on the job training
- ✓ Meeting established quality and efficiency requirements
- ✓ Attendance to relevant meetings/classes

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We have discussed the procedures involved in instrument reagent quality control but it should be stressed that the most important part of the whole process are staff. For the whole system to work efficiently we need to invest in our staff to ensure that they reach a level of competence that enables them to undertake their job role effectively and efficiently. The educational component of medical technologists cannot be stressed high enough and training is the key to staff understanding the data generated and how to obtain maintain a quality service and generate robust and quality data.

# Training

- ✓ Standardize on the job training
  - ✓ Written training goals
    - ✓ Pipetting accuracy and precision
    - ✓ Testing accuracy and precision
    - ✓ Recognize problems and special situations
    - ✓ Troubleshooting common problems
  - ✓ Qualified trainers
  - ✓ Training checklists for each task/assay
  - ✓ Consistency in lesson plans
  - ✓ Trainee and trainer sign-off

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Staff should not only undertake on the job training which will hone the skills of data acquisition and analysis but there should be a scheduled and monitored training programme that may involve off site education.

## Competency Assessment

- ✓ An objective evaluation that assures a person *continues* to perform job assignments accurately, proficiently, and according to established standards.
  - ✓ New staff semi-annual/existing annual
  - ✓ Assessed for all tasks/job assignments
  - ✓ Assessment performed by qualified staff
  - ✓ Objective predefined criteria
  - ✓ Documented

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All staff should record their competency assessments which are duly documented and on-going. The staff should have clear learning objectives and these should be reviewed on a frequent basis.

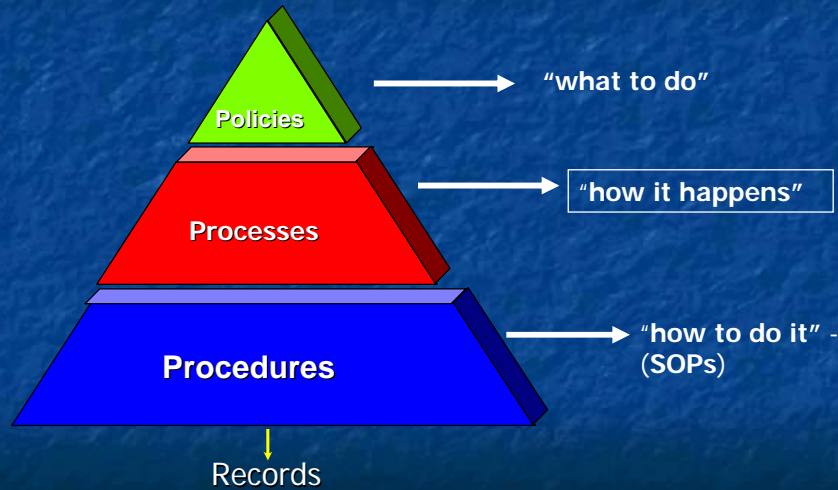
# Competency Assessment

- ✓ Direct Observation
  - ✓ Watch test performance
  - ✓ Compliance to SOP
- ✓ Records review
  - ✓ QC maintenance records
- ✓ Test performance
  - ✓ Blind testing
  - ✓ Proficiency testing (EQA)
- ✓ Problem solving
  - ✓ Quizzes
  - ✓ Trouble shooting

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In order to monitor competency a variety of methods and tools can be used. These should encompass all aspects of laboratory practice and logged by the trainee in their portfolio. This portfolio should be regularly inspected both internally and externally.

# Policies and Procedures

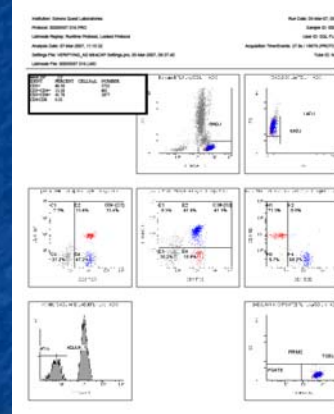


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To help the laboratory achieve the goal of raising standards (continually) there should be a set of policies and procedures available to ALL staff. This will ensure that everyone is able to identify the goals of the department and also ensures that everyone is working in the same manner (as previously outlined). Here we can see the difference between , policies, processes and procedures. It is generally management that define the policies and processes such that appropriate SOPs are designed and written that will enable the department and the staff therein achieve their goals.

# Records

- ✓ Quality Control
- ✓ Non-conformances
- ✓ Corrective Action
- ✓ Equipment Records
- ✓ Training/Competency
- ✓ Validation
- ✓ Reports and records
- ✓ Proficiency testing



**"If you didn't document, it didn't happen"**

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However, one of the most important aspects of Policies, Processes and Procedures is the record keeping. It is vitally important that everything from quality control to training to proficiency testing is suitably recorded and retained should an untoward incident occur. This will then allow the department and individual(s) to learn from their experiences.

## Inspection/Audits

- ✓ Can be based on complaints or proactive
- ✓ External (or internal) experts verify lab's conformance to set of predefined set of standards.
- ✓ Non-conformances are documented and corrected.
- ✓ Unannounced audits are best to ensure each lab is always "audit ready"

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However, one of the most important ways a laboratory can learn from their practices is to undertake regular audits. These audits should be both vertical (observing the whole process from start to finish) and horizontal (looking at a specific part of the process and then ascertaining what happens to a number of specimens or results within that part). The best way to undertake audits is usually unannounced as this ensures the laboratory is always audit ready but it ensures that the laboratory is also compliant with the standards laid down by external agencies who will accredit the laboratory. Undertaking audits will help reveal deficiencies in a system for example who and how often refrigerator temperatures are checked and recorded.

# Quality Improvement

- ✓ Understand customer requirements
  - ✓ Turnaround time
  - ✓ Shipping/Transport errors
  - ✓ Errors in reporting
  - ✓ Sub optimal samples – unable to test
- ✓ Set target threshold
- ✓ Measure and improve
- ✓ Document improvement

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However, it the laboratory should never lose sight of thee fact that they are there to serve the customer and that they thoroughly understand the customer requirements. This in turn will lead to ensuring the service is continually improving.



# Summary

- ✓ Quality Assurance (QA) controls pre-analytic, analytic and post analytic activities
- ✓ Quality Control (QC) monitors and maintains assay specifications (precision and accuracy) over time.
- ✓ Goal meet customer expectations for quality through continuous quality improvement (CQI).

**"IF IN DOUBT - REPEAT"**

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To summarise both IQC and EQA should be used in tandem to complement each other such that the laboratory achieves continuous quality improvement and thereby improve patient care. However, one of the most important messages to take from this slide show is – IF IN DOUBT - REPEAT