

Division of Blood Transfusion Services

Ministry of Health and Family Welfare



Quality Management - TTI



Teaching Aim

- To familiarize participants with essential quality elements that govern TTI tests such as validation of test run, types of control used and maintenance of equipments.



Why we require Quality in TTIs testing

- Blood is a source of TTIs (14 new viruses in the past 28 years-
one new virus every two years!!)
- Quality is Essential to prevent TTIs
- Lack of quality can result either in TTI or wastage of blood
- TTI testing is dependent on number of variables which need to be controlled



Essential elements governing quality in TTI

- Quality of the specimen used for testing
- Quality of kits used for testing
- Quality & Calibration of equipment used
- Use of SOPs for testing
- Type of Controls used while testing
- Interpretation of results
- Validation of results
- Record keeping
- Training of staff in SOPs



The quality of specimen used for testing

- Specimen should be properly labeled
- Specimen should be clear and sterile
- Lipemic, hemolysed and contaminated specimens do not yield reliable results
- Bio-safety measures are very crucial to prevent laboratory infections - handling and disposal.
- Avoid adding preservatives - some interfere with test results



Quality of kits used for testing

- In general kits with highest sensitivity and specificity should be used in a BTS for TTI testing
- All kits and reagents should be used within the expiration date
- Kits which are used should have approval of certifying authority(DCGI,NACO)
- Never interchange reagents from one kit to another or one lot to another



Quality and calibration of equipments used

- Always use standard equipment in a BTS for TTI testing - ELISA reader & washer, Micro-pipettes, incubators, shakers etc.
- Periodic calibration of equipment is vital to maintain quality -
- Periodic servicing of equipment is crucial for optimal use of equipment- ELISA washers
- Proper documentation on equipment check and its performance is essential- maintain records



Controls used in the assay for testing

- **Internal kit controls** : Include the positive control, Negative control. At times may include a calibrator provided by the manufacturer
- **External Controls:** Include positive samples from the laboratory either pooled or single, diluted or undiluted. Essential to incorporate this to monitor quality in testing procedures.
- **Intra-run and Inter-run reproducibility** : three slots per run and on three consecutive days.



Validation

- It is assuring that a system, process or equipment is performing the way it is supposed to do.



Validation Tools

- Include positive and negative controls in every test run
- Include additional validation measures where possible e.g.
 - Rapid tests with internal control, spot or line
 - Independent readings of rapid tests by 2 people
 - Use of mechanical readers if available - to reduce subjectivity



Interpretation of test results

- As a rule, all readings (both quantitative and qualitative) and calculations should be checked by two individuals
- Validation of every run is essential for proper interpretation of results
- Proper records - print outs of results, calculations of cut off values, graphs, etc. should be maintained
- Any errors detected should be brought to the notice of the concerned staff and corrective measures instituted promptly



Controls

- **Internal controls**

- Set of controls (Positive & Negative) provided along with the kit
- To be used only in those batches of kit from which they originate
- The internal controls do not detect minor deterioration of kits



Controls (contd..)

- **External controls**
 - Set of controls included from outside
 - Positive (Borderline Reactive) & Negative
 - Detect minor error in the assay performance

Sources of External Controls

- National reference laboratories
- Commercial control panels
- In-house prepared external controls



Preparation of In-house External Controls

NACO Guidelines

Select sero-reactive serum/plasma



Retest the sample with another kit



Heat inactivate the sample **@ 56°C X 30 min**

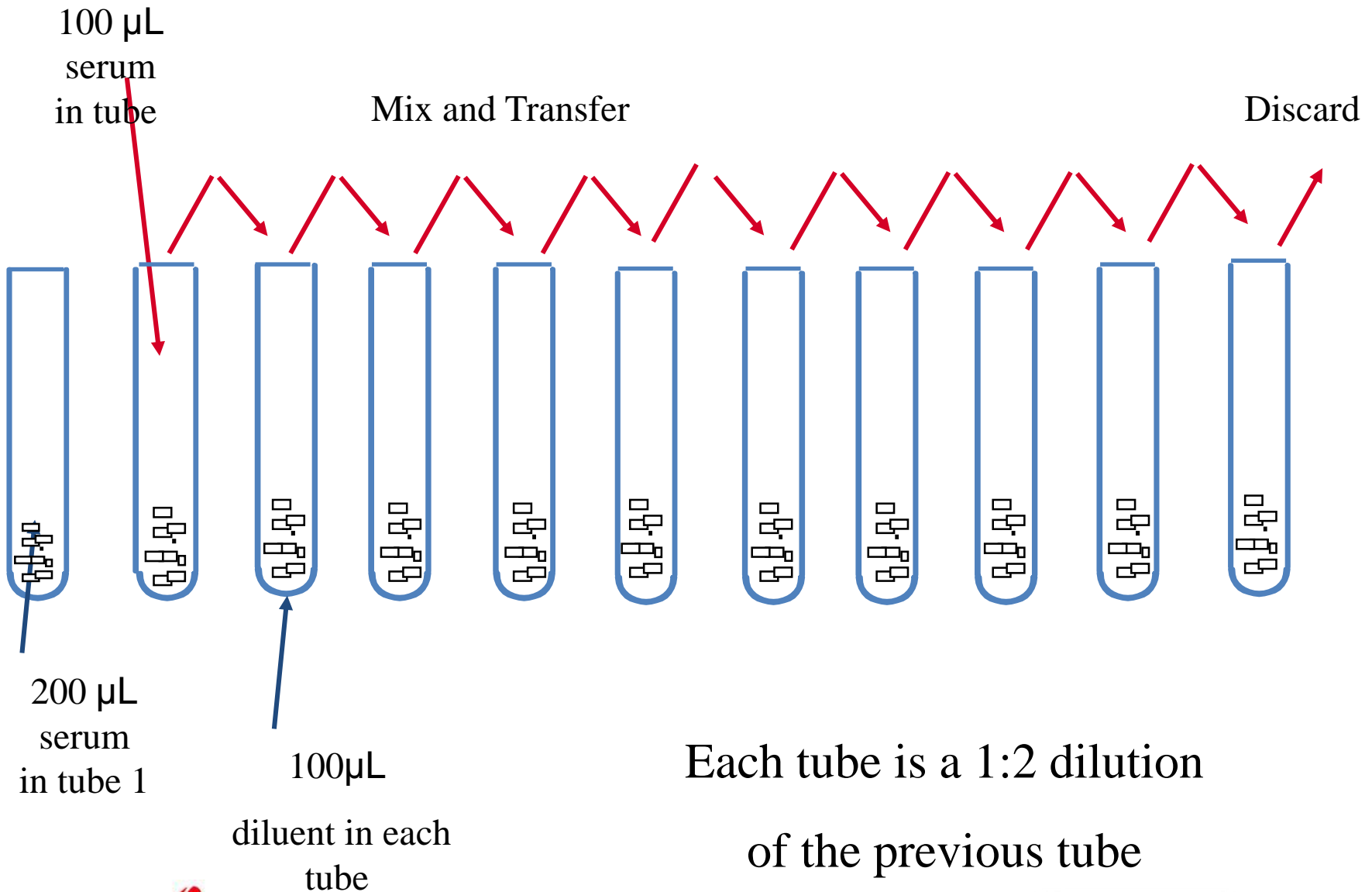


If plasma taken, **re-calcify** it to obtain serum



Make **serial dilution** of the sample with a sero-negative serum

Making Suitable Dilutions



Preparation of In-house External Controls (contd...)

After test run, calculate **ER** for each dilution **ELISA ratio**
= sample OD / cut off OD



Select the dilution with ER b/w **1.5 to 2**



Prepare external control **aliquots** of dilution selected above



Store at -20°C or below **@ 1 year**



Once thawed, control can be kept **@ $2-8^{\circ}\text{C}$, 1 wk**



Need for E ratio

- Cut off value of the run depends on the principle of the test , manufacturer guidelines and recommended protocol for the calculation.
- Some degree of variation in internal controls (use of kits from same manufacturer).
- Results in the variation in the cut off values
 - Incubation period
 - Preparation of reagents
 - Plate to plate and well to well variation (antigen coating)



- OD of the controls would expectedly influence the OD value of the test samples in similar directions.
- Relative reactivity of the given sample and the cut off would not vary.
- This relative reactivity in a particular run is expressed and termed as **E ratio**. (ratio between the OD of sample and cut off)

$$\text{E ratio} = \frac{\text{OD value of external control}}{\text{OD value of cut off}}$$



Preparation of QC charts/Levy Jennings charts

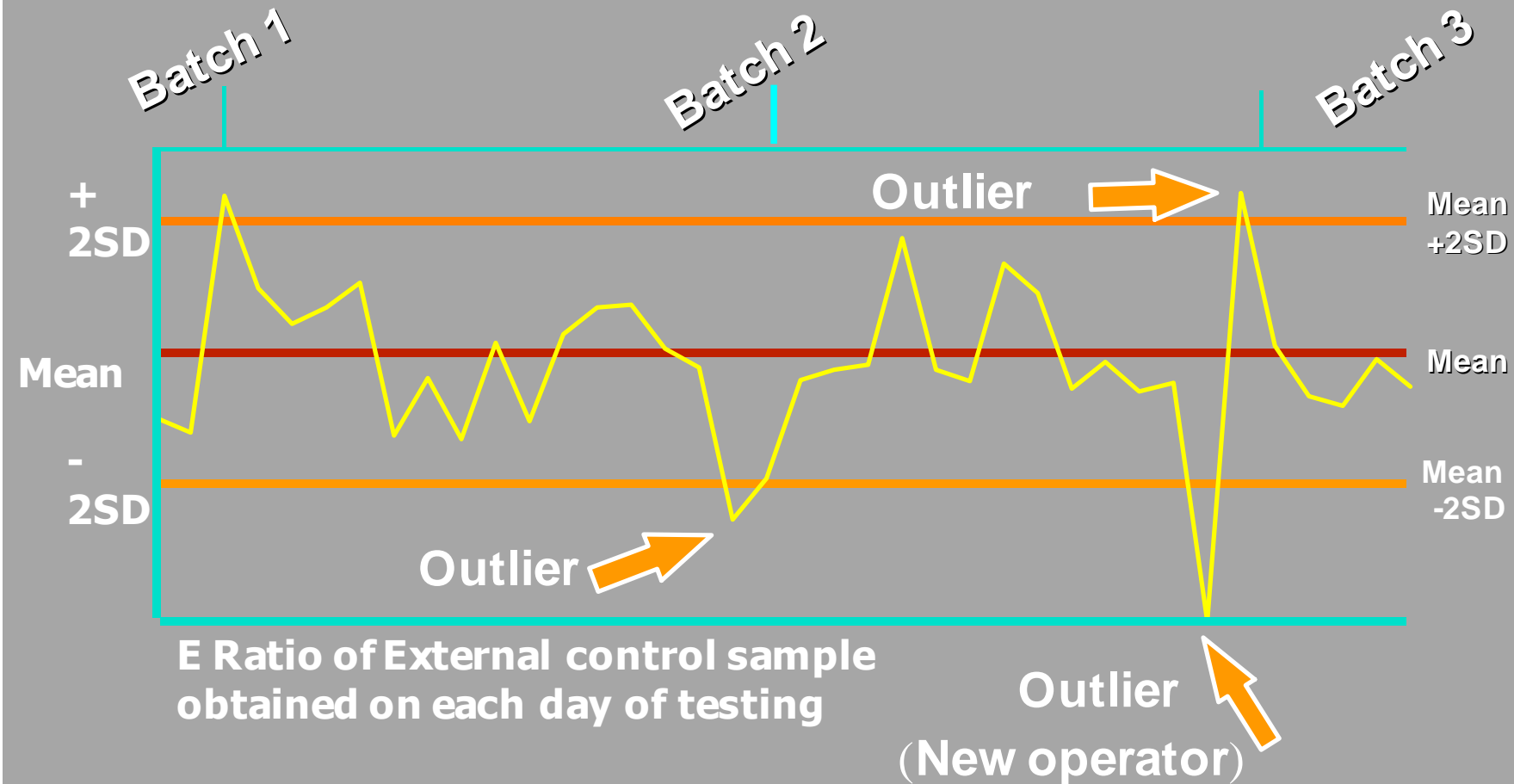
- E ratios are plotted on the Y axis in chart and consecutive dates of runs are plotted on X axis.
- Draw the limit of 2SD on either side of the mean
- Include at least 30 runs on the same graph
- Mean and $\pm 2SD$ plotted on the graph.
- Change of operator and batch of assay should be recorded



$$SD = \sqrt{Variance}$$

$$Variance = \frac{\sum (X - \bar{X})^2}{n-1}$$

Levy-Jennings Control Chart



L J chart –Scope and application

Detection of the following

- Systematic variation
- Random variation
- Lot to lot variation
- Day to day variation



Applications of control charts

- Highlight the outliers (values outside ± 2 SD)
- Reveal batch to batch variation
- Reveal operator to operator variation
- Changes in assay performance even when test runs are valid



Systematic Variation

- **Trend**-Results change gradually in either direction indicating slowly changing parameters-deteriorating reagents, equipment failing
- **Shift**-Results fall sharply on one side of the mean indicating a major change has occurred

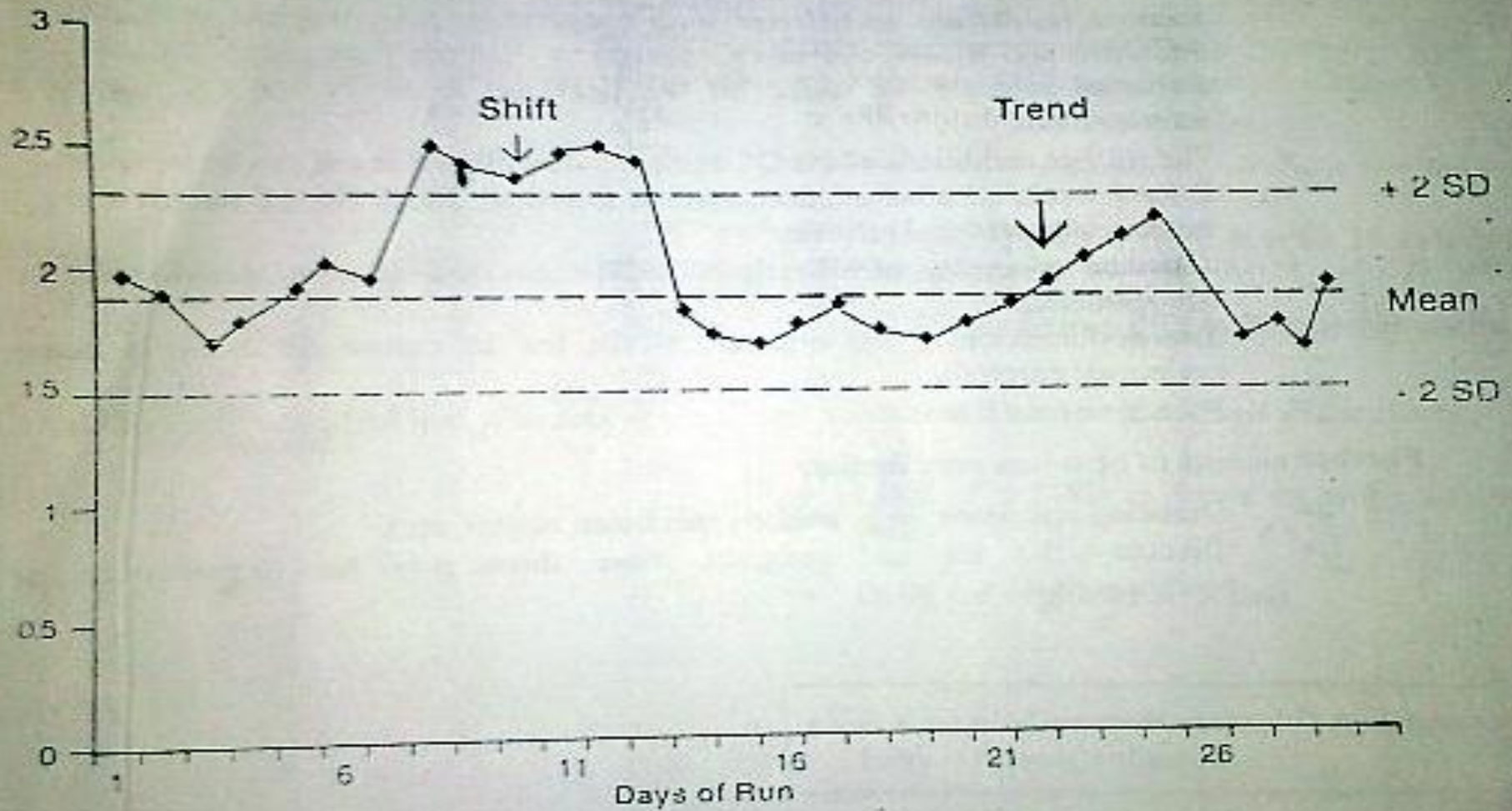


Random Variation

- Observance of one result, significantly different from other results without any pattern
- **Causes**
 - Transcription errors
 - Sample mix-up
 - Poor pipette precision
 - Poor mixing of samples
 - Reader not calibrated
 - Washing inconsistent



Chart showing shift and trend



Interpretation of aberrant results

- Control values of six consecutive runs fall on one side of mean(SHIFT)
 - Switching to new lot of kits
 - New reagents
 - Changes in incubation temperature
 - New technical hand
- Six consecutive points distributed in on general direction (TREND)
 - Deterioration of reagents
 - Slowly faltering equipment.

Quality control of equipment.

- Quality Control
 - Frequency – monitored periodically
 - Parameters
- Documentations
 - SOP – numbering
 - quality control
 - log books



ELISA Reader



ELISA Reader (contd...)

- Photometric instrument
- OD of special plates & standard colour solution are recorded
- filter should be protected from moisture and fungal growth
- keep silica gel packs in the filter box
- Calibration is done every six months(supplier)
- Results of OD should be within 10% of expected
- Daily check – negative & positive controls added to each run



ELISA Washer

After Use-

- Fill the rinse bottle with about 500 ml of distilled water.
- Dispose off the unused wash buffer. Rinse with distilled water, a couple of times and leave about 500 ml in the wash bottle.
- Fix the cap tightly.



Water baths & Incubators

- Daily recording of temp. using a calibrated thermometer
- Acceptable results are the expected temp. \pm a narrow range ($\pm 0.5^{\circ}\text{C}$) predetermined by the laboratory
- Distilled water changed regularly
- Bacterial cultures – periodically



Pipette



Pipette (contd...)

Quality control of pipettes

- All items at ambient room temp.
- Record the weight of empty beaker
- Record the temp. of tube filled with distilled water
- Pipette a known volume of water (expected volume)
- Record:

[wt of beaker + water] – wt of empty beaker

= weight of water



Pipette (contd...)

- Delivered vol. =
$$\frac{\text{weight of water}}{\text{temp. factor} \times \text{sp. gravity of water}}$$
- Repeat this 10 times, changing the tip
- Calculate mean, SD and CV
- % Deviation =
$$\frac{(\text{expected vol.} - \text{delivered vol.}) \times 100}{\text{expected vol.}}$$
- ✓ % deviation < 1.5%
- ✓ CV < 1%



Percent deviation

- **Percent deviation =**

$$\frac{[(\text{Expected value} - \text{observed value}) \times 100]}{\text{Expected value}}$$



Equipment	Monitoring frequency	Frequency of calibration
Refrigerator	Daily	As often as necessary
Refrigerated centrifuge	Each day of use	On installation, after repairs & annually
Deep freezer	Daily	As often as necessary
Table top centrifuge	Daily	Tachometer, every 6 months
Water bath	Daily	As often as necessary
Autoclave	Each time of use	As often as necessary
Platelet agitators	Daily	On installation, after repairs & annually
Blood collection monitor	Daily	On installation, after repairs & annually