

Analytic Phase – Quality at the Bench

This session focuses on the analytic phase of testing—from specimen preparation through data interpretation. Advances in instrumentation and automation have reduced error rates, but challenges like sample interference and test validation remain. The lecture outlines CLIA-mandated policies, training, QC practices, and instrument verification requirements.



Learning Objectives



Describe the analytic phase and its boundaries within the testing cycle.



Recognize sources of analytic error, such as interfering substances and improper sample prep.



List the CLIA regulations applicable to test method verification, calibration, QC, and instrument maintenance.



Apply tools like pro formas, QC trend plots, and SOPs to ensure consistency and traceability in test results.

Introduction to the Analytic Phase

Time Allotted: 10 minutes

Definition:

The **analytic phase** begins when a specimen is received in the lab and prepared for testing, and ends when the raw test result is generated, interpreted, and validated by authorized personnel.

Activities in this Phase:

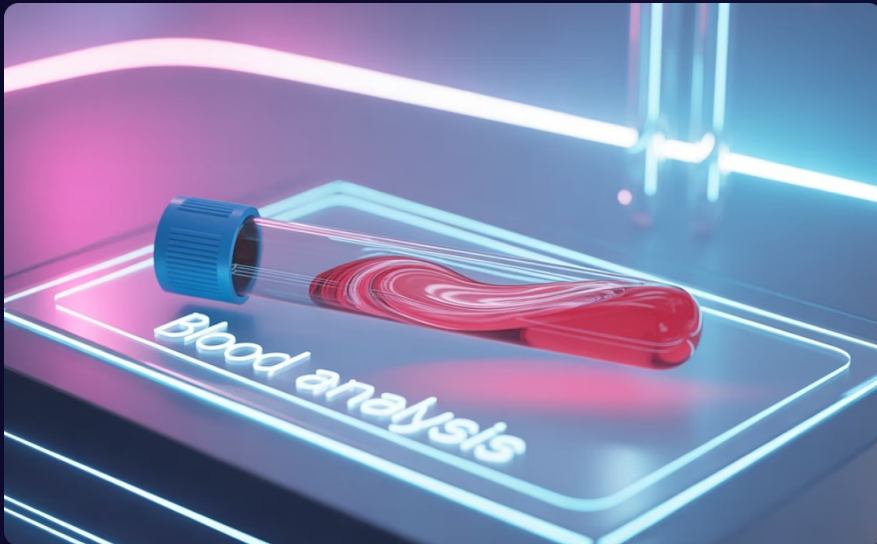
Specimen **preparation**, centrifugation, aliquoting

Actual **instrumentation** and biochemical/clinical analysis

- Result validation and initial data entry into LIS



Common Sample Issues



Hemolysis

Lysed red blood cells falsely elevate potassium, LDH, AST



Lipemia

Turbidity affects photometric readings



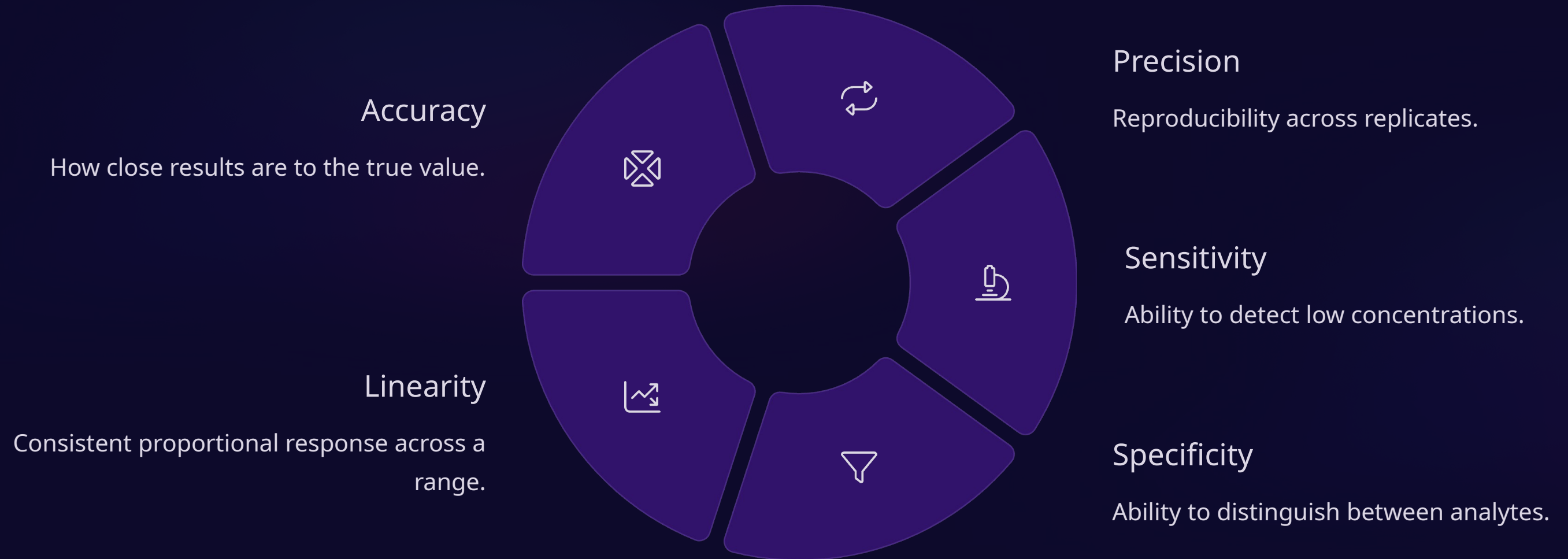
Icterus

Bilirubin can interfere with colorimetric assays

Discussion Prompt: What pre-analytic issues have you seen impact the analytic phase?

Key Performance Characteristics

CLIA-mandated



Establishing Performance

Required when:

- **Introducing new assays**
- **Validating manufacturer claims**
- **Modifying FDA-cleared methods**

Must **compare lab-specific performance** to reference standards or manufacturer data.

Documentation Required:

- Linearity verification with known standards
- Replicate runs to establish intra- and inter-assay variability
- Spiked/recovery studies for specificity

Best Practice Tip: Document all validation studies in a **Test Performance Verification Binder** organized by assay.



CLIA Requirements for the Analytic Phase

CLIA-Mandated Activities

1 Written SOPs & Policy Manuals:

- Must describe all steps of the testing process and be reviewed/approved by lab director.
- Must include test limitations, safety protocols, reagent handling.

3 Proficiency Testing (PT):

Must participate in **CMS-approved PT** programs.

Use of **alternative assessment** when PT is not available.

2 Training & Competency:

Staff must be **trained before** performing tests.

Competency must be **assessed semi-annually** for new employees, then annually.

4 Instrument Calibration & Maintenance:

Calibration and function checks **daily, weekly, monthly** as appropriate.

Service logs must include manufacturer, service date, and validation post-maintenance.

Instrument Calibration & Maintenance



Proper instrument maintenance is critical for ensuring accurate and reliable test results. CLIA requires:

- Regular calibration according to manufacturer specifications
- Function checks at specified intervals (daily, weekly, monthly)
- Comprehensive service logs documenting all maintenance activities
- Validation of instrument performance after any maintenance

Interactive Prompt: What documentation does your lab keep for each analyzer?

Pro Forma Worksheets

A **pro forma** is a fillable form used during real-time testing.

Should capture:

- **Reagent lot numbers**
- **Calibrators and QC material**
- **Instrument ID**
- **Analyst performing the test**
- **Incubation times/temperatures**
- **Sample integrity observations**



An abstract background graphic on the left side of the slide. It features a dark blue grid with glowing spheres in shades of pink, orange, and blue. Lines connect some of the spheres, creating a network-like structure. The overall aesthetic is futuristic and data-oriented.

Daily QC Checks

Run QC Samples

Use **two levels of QC** (high and low) with each batch of patient samples.

Compare Results

Compare QC results to expected ranges established during validation.

Plot on Charts

Plot results on **Levy-Jennings charts** to visualize trends over time.

Identify Issues

Identify trends or shifts before patient results are impacted.

Levy-Jennings Charts

Visual Demo



Key components of a Levy-Jennings chart:

Mean line - represents the expected value

1 SD, 2 SD, 3 SD rules - standard deviation boundaries

Westgard rules for detecting systematic errors:

- 1:2s - one control exceeding 2 SD
- 2:2s - two consecutive controls exceeding 2 SD
- R:4s - difference between two controls exceeding 4 SD

Bioassays & Special Considerations

Special quality control procedures are required for:

Media lots used in cell culture

Consumables that may impact test results

Plasticware in IVF labs

Some manufacturers provide certificate of analysis; others require in-house testing.



Corrective Action Protocol



Document QC Failure

Record all details of the quality control failure including date, time, test, and observed values.



Investigate Root Cause

Perform root cause analysis (RCA) to determine the source of the error.



Take Corrective Action

Include repeat testing, equipment check, or reagent replacement as needed.



Obtain Sign-off

Sign-off by supervisor or lab director required before resuming testing.

Case Study 1: QC Failure

Scenario:

QC fails in the morning for your progesterone assay. The high control is above 3 SD.

Action Steps:

- Quarantine the test results for that day.
- Check calibrator lot number and pipetting technique.
- Review instrument logs for temperature drift.



Precision.
Innovation.
Assurance.

Case Study 2: Technical Error

Scenario:

A technician forgets to pre-warm media before sperm prep, and motility is severely reduced.

Discussion:

- Was this a technical issue or a training gap?
- How should this be documented and reviewed?



Sources of Analytic Error



Interfering Substances

Hemolysis, lipemia, and icterus can all interfere with accurate test results by affecting photometric readings or chemical reactions.



Environmental Factors

Temperature fluctuations, humidity, and light exposure can affect reagent stability and reaction kinetics.



Improper Sample Prep

Incorrect centrifugation, inadequate mixing, or improper aliquoting can lead to inaccurate results.

Instrument Verification Requirements

Initial Verification:

- Accuracy verification against reference standards
- Precision testing with replicate samples
- Linearity assessment across the reportable range
- Analytical measurement range verification

Ongoing Verification:

- Daily quality control checks
- Periodic calibration verification
- Proficiency testing participation
- Post-maintenance verification



Quality Control Documentation Best Practices



Standardized Forms

Use consistent documentation formats for all QC activities to ensure completeness and facilitate review.



Electronic Records

Maintain searchable electronic records with appropriate security and audit trails when possible.



Regular Review

Schedule periodic review of QC documentation by supervisors and the laboratory director.



Retention Policies

Establish clear retention policies that comply with CLIA requirements and institutional policies.





Training and Competency Assessment

Initial Training:

- Comprehensive review of test principles
- Hands-on demonstration of procedures
- Supervised practice runs
- Knowledge assessment before independent testing

Competency Assessment:

- Direct observation of routine test performance
- Monitoring the recording and reporting of results
- Review of intermediate test results or worksheets
- Assessment of test performance through testing unknown samples
- Assessment of problem-solving skills

Summary: Ensuring Quality in the Analytic Phase

Standardized Procedures
Implement comprehensive SOPs for all testing processes

Error Investigation
Promptly investigate and document all quality control failures



Proper Training

Ensure all staff are thoroughly trained and regularly assessed

Rigorous QC

Maintain strict quality control practices with proper documentation

Regular Maintenance

Follow manufacturer guidelines for instrument calibration and maintenance

The analytic phase requires careful attention to detail and strict adherence to established protocols to ensure accurate and reliable test results for optimal patient care.