Spectroscopy in Diagnostics

Clinical Chemistry

Automated analyzers. Glucose, electrolytes, enzymes.

Immunoassays

ELISA, CLIA. Antibody-based detection. High sensitivity.

Molecular Diagnostics

PCR, qPCR, NGS. Pathogen detection, cancer markers.

Validation

Accuracy, precision, sensitivity, specificity. FDA/CLIA requirements.

1. Clinical Chemistry

Overview
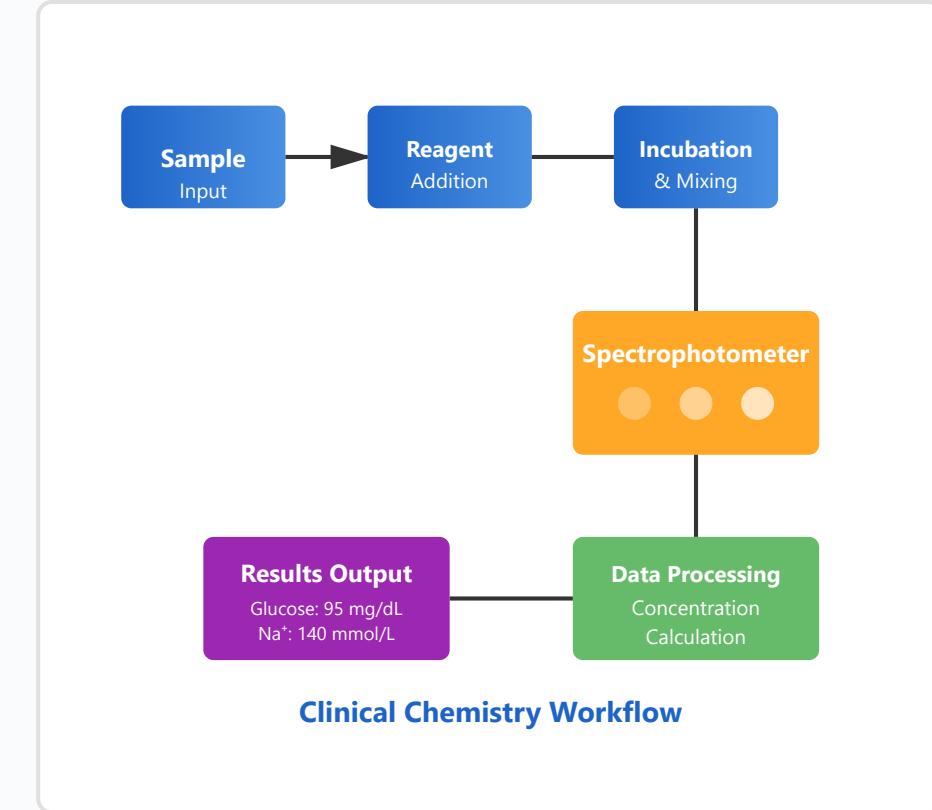
Clinical chemistry utilizes spectroscopic techniques to quantify biochemical substances in body fluids. Modern automated analyzers process hundreds of samples per hour using various spectroscopic methods.

Key Techniques

- **UV-Visible Spectroscopy:** Measures absorbance at specific wavelengths for colorimetric assays

- **Photometry:** Quantifies enzyme activities through substrate-product reactions

- **Ion-Selective Electrodes (ISE):** Measures electrolytes (Na^+, K^+, Cl^-, Ca^{2+})

Common Applications

- Blood glucose monitoring (diabetes management)

- Liver function tests (ALT, AST, bilirubin)

- Kidney function tests (creatinine, BUN)

- Lipid panels (cholesterol, triglycerides)

- Electrolyte balance assessment


```
Sample          Reagent          Incubation
Input           Addition         & Mixing

                                 Spectrophotometer
                                 ● ● ●

Results Output              Data Processing
Glucose: 95 mg/dL           Concentration
Na⁺: 140 mmol/L             Calculation
```

Clinical Chemistry Workflow

2. Immunoassays

Overview

Immunoassays leverage antigen-antibody interactions combined with spectroscopic detection to identify and quantify specific analytes with exceptional sensitivity and specificity.
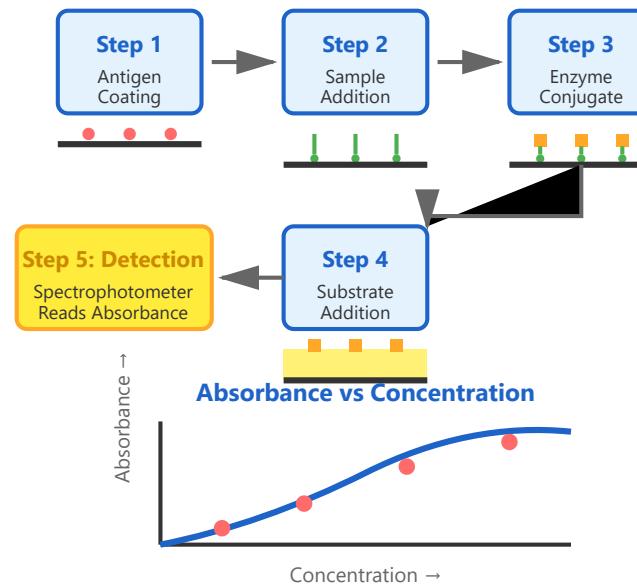
Major Techniques

- **ELISA (Enzyme-Linked Immunosorbent Assay):** Uses enzyme-substrate reactions producing colored products measured spectrophotometrically

- **CLIA (Chemiluminescence Immunoassay):** Measures light emission from chemical reactions, offering higher sensitivity

- **Fluorescence Immunoassays:** Utilize fluorescent labels for detection at very low concentrations

Clinical Applications

- Hormone level testing (thyroid, reproductive hormones)

- Tumor marker detection (PSA, CEA, CA-125)

- Infectious disease screening (HIV, hepatitis)

- Allergy testing (IgE antibodies)

- Autoimmune disease diagnosis

- Drug monitoring and toxicology

ELISA Process

| Step 1 Antigen Coating | Step 2 Sample Addition | Step 3 Enzyme Conjugate |

Step 5: Detection — Spectrophotometer Reads Absorbance

Step 4 Substrate Addition

Absorbance vs Concentration

Absorbance ↑ / Concentration →

3. Molecular Diagnostics

Overview

Molecular diagnostics analyzes biological markers in the genome and proteome using spectroscopic and fluorescence-based techniques to detect genetic variations, pathogens, and disease markers at the molecular level.

Core Technologies

- **PCR (Polymerase Chain Reaction):** Amplifies specific DNA sequences for detection

- **qPCR (Quantitative PCR):** Real-time fluorescence monitoring of DNA amplification

- **NGS (Next-Generation Sequencing):** Massive parallel sequencing with fluorescent detection

- **Fluorescence In Situ Hybridization (FISH):** Visualizes genetic material in cells
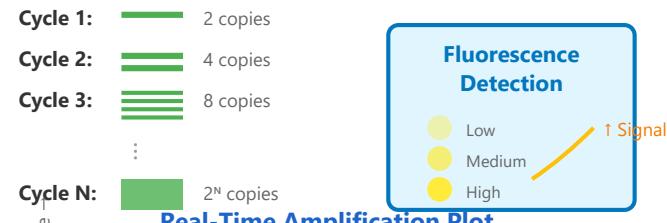
Clinical Applications

- Infectious disease identification (COVID-19, influenza, tuberculosis)

- Cancer genomics and personalized oncology

- Genetic disorder screening and prenatal testing

- Pharmacogenomics for drug response prediction

- Minimal residual disease monitoring

- Transplant compatibility testing
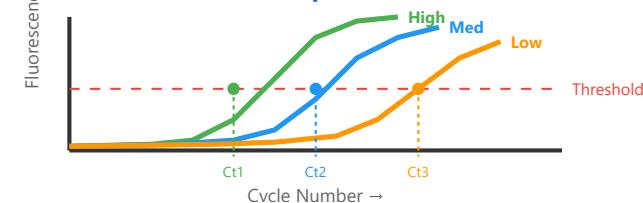
Real-Time PCR (qPCR) Process

DNA Sample — Target Sequence → PCR Mix Added — Primers, Polymerase, Fluorescent Dye → Thermal Cycler — 95°C→60°C→72°C

Amplification Cycles

- Cycle 1: 2 copies
- Cycle 2: 4 copies
- Cycle 3: 8 copies
- Cycle N: 2^N copies

Fluorescence Detection
- Low
- Medium
- High
- ↑ Signal

Real-Time Amplification Plot

Fluorescence — High, Med, Low — Threshold

Ct1 — Ct2 — Ct3

Cycle Number →

4. Validation

Overview

Analytical validation ensures that diagnostic tests produce accurate, reliable, and reproducible results. Regulatory compliance with FDA and CLIA standards is essential for clinical implementation.

Key Performance Metrics

- **Accuracy:** Closeness of measured value to true value (assessed using reference materials)

- **Precision:** Reproducibility of results (intra-assay and inter-assay variability)

- **Sensitivity:** Ability to correctly identify positive cases (True Positive Rate)

- **Specificity:** Ability to correctly identify negative cases (True Negative Rate)

- **Limit of Detection (LOD):** Lowest detectable concentration

- **Limit of Quantification (LOQ):** Lowest accurately quantifiable concentration

Regulatory Requirements

- **FDA Approval:** Required for in vitro diagnostic devices

- **CLIA Certification:** Laboratory quality standards

- **Quality Control:** Daily calibration and control samples

- **Proficiency Testing:** External quality assessment programs

Validation Performance Metrics

Confusion Matrix

	Disease +	Disease -
Test +	**True Positive** (TP) Correctly identified	**False Positive** (FP) Type I Error
Test -	**False Negative** (FN) Type II Error	**True Negative** (TN) Correctly rejected

Quality Control
- ✓ Daily calibration
- ✓ Control samples
- ✓ Proficiency testing
- ✓ Equipment maintenance
- ✓ Staff training

Performance Calculations

Sensitivity
TP / (TP + FN)

Specificity
TN / (TN + FP)

Accuracy
(TP + TN) / Total

Precision (PPV)
TP / (TP + FP)

Regulatory
FDA Approval
CLIA Certification