

Introduction to Molecular Diagnostics

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Key concepts, workflow, technologies, and clinical applications

1. What Is Molecular Diagnostics?

Aspect	Description
Definition	Use of nucleic acid–based techniques to detect, quantify, or characterize DNA, RNA, or related molecules in a patient sample.
Goal	Provide rapid, precise, and often quantitative information about genetic alterations, infectious agents, or biomarkers that guide diagnosis, prognosis, and therapy.
Scope	- Infectious disease testing (viral, bacterial, fungal) - Genetic disease screening (carrier, prenatal, newborn) - Oncology (mutation profiling, minimal residual disease) - Pharmacogenomics (drug response prediction)

2. Core Principles

1. **Target Nucleic Acid** – DNA, RNA, or cDNA derived from the sample.
 2. **Amplification** – Generation of enough copies for detection (e.g., PCR, isothermal methods).
 3. **Detection/Readout** – Fluorescence, luminescence, electrophoresis, sequencing, or hybridization signals.
 4. **Interpretation** – Bioinformatic or manual analysis to translate signal into a clinical result.
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3. Typical Workflow

1. Pre-analytical Phase

- Sample collection (blood, plasma, swab, tissue, urine).
- Preservation & transport (temperature control, nucleic acid stabilizers).
- Nucleic acid extraction (spin columns, magnetic beads, automated platforms).

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2. Analytical Phase

- **Amplification** (PCR, RT-PCR, LAMP, RPA).
- **Target enrichment** (primer/probe design, multiplexing).
- **Detection** (real-time fluorescence, melt-curve analysis, microarray, NGS).

3. Post-analytical Phase

- Data processing (quality control, threshold setting).
- Result validation (controls, repeat testing if needed).
- Reporting (standardized format, clinical interpretation, recommendations).

4. Major Technologies

Technology	Principle	Typical Turn-around	Main Uses
Conventional PCR	Thermal cycling amplifies DNA	2–4h	Pathogen detection, genotyping
Real-time qPCR (RT-qPCR)	Fluorescent probes monitor amplification in real time	1–2h	Viral load, gene expression
Isothermal Amplification (LAMP, RPA)	Amplification at a single temperature	<30min	Point-of-care, low-resource settings
DNA Microarrays	Hybridization of labeled targets to thousands of probes	4–6h	SNP panels, pathogen panels
Next-Generation Sequencing (NGS)	Massive parallel sequencing of millions of fragments	1–3days (clinical panels)	Comprehensive mutation profiling, metagenomics
Digital PCR (dPCR)	Partitioned reactions enable absolute quantification	2–4h	Low-frequency mutation detection, copy-number analysis

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CRISPR-based Diagnostics (e.g., SHERLOCK, DETECTR)	Cas enzymes cleave reporter upon target binding	<1h	Rapid viral detection, field testing

5. Clinical Applications

5.1 Infectious Diseases

- Pathogen identification – SARS-CoV-2