

Recent Advances in Hormone Measurement

Neha Rani Verma, Seema Shah, Suprava Patel, Rachita Nanda, Eli Mohapatra

Hormones play a crucial role in various physiological functions of body. They act as signaling molecules and are secreted by various glands. A minor imbalance in hormones leads to serious health implications, like diabetes, infertility, thyroid disorders, growth abnormalities etc. Analysis of hormone level is important to identify different diseases. Traditional methods for the hormone measurement are Radioimmunoassay (RIA) and Bioassay. Enzyme linked immunosorbent assay (ELISA), Fluoroimmunoassay (FIA), and Chemiluminescence immunoassays CLIA are techniques, which are commonly used now a days. These methods are based on antigen antibody reactions and used in many labs. Limitations of these methods are cross reactivity with structurally similar molecules, interference due to difference in biological matrix interference. Recent techniques like mass spectrometry, biosensors and microfluidics are effective techniques with enhanced selectivity and specificity and decrease interference.

Keywords: *Thyroid disorders, Diabetes, Mass spectrometry, Biosensors, Radioimmunoassay (RIA)*

Neha Rani Verma¹, Seema Shah^{2*}, Suprava Patel³, Rachita Nanda³, Eli Mohapatra⁴

¹Assistant professor, Department of Biochemistry, AIIMS Raipur, India.

²Additional professor, Department of Biochemistry, AIIMS Raipur, India.

³Professor, Department of Biochemistry, AIIMS Raipur, India.

⁴Professor and HOD, Department of Biochemistry, AIIMS Raipur, India.

*Email: sshah@aiimsraipur.edu.in

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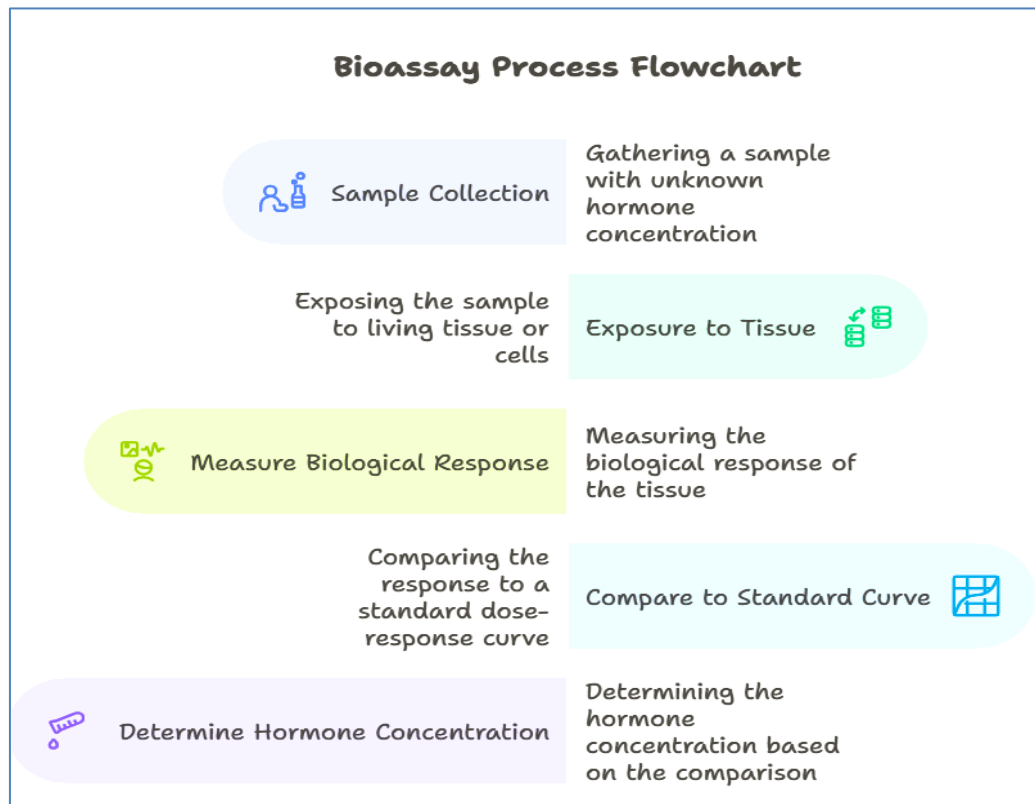
Introduction

Hormones serve as essential chemical messengers in the human body, regulating a wide range of physiological functions including growth, metabolism, reproduction, and stress response. Accurate measurement of hormone levels is essential in clinical endocrinology for the diagnosis, treatment and monitoring of a number of endocrine disorders such as diabetes, thyroid diseases, adrenal insufficiencies, and reproductive dysfunctions. Historically, hormone measurement was done using bioassays, which are less sensitive and challenging because they depended on biological reactions to hormones. Over the decades, technological advancements have driven hormone assay methods from these crude methods to highly sensitive immunoassays and advanced mass spectrometry, improving not only the diagnostic accuracy also

enabling earlier detection of endocrine abnormalities. The clinical relevance of precise hormone measurement lies in its ability to guide the clinicians in taking therapeutic decisions, monitor treatment efficacy, and predict disease prognosis. For example, thyroid-stimulating hormone (TSH) assays are essential in managing thyroid diseases, while insulin and C-peptide measurements guide the treatment of diabetes. Emerging techniques further promise improved specificity, reduced turnaround time, and the ability to measure multiple hormones simultaneously, which is a prerequisite in complex disorders involving multiple hormonal axes. This chapter will explore the development of hormone measurement techniques from past to present, highlighting the role of emerging technologies on clinical practice, to bridge foundational knowledge with cutting-edge laboratory applications.

Bioassay

Bioassay is one of the earliest methods used for hormone measurement. With this approach the hormone concentration is evaluated by a biological reaction, such as change in metabolism, contraction of muscle etc. Bioassays offer functional measurement of hormone activity rather than just concentration. However, in comparison to newer technique they are time-consuming, require living organisms or tissues, and are less specific and sensitive.



Advantages

- **Functional assessment:** determine the actual biological activity of the hormone, not just its concentration and provide insight into its physiological effect.
- **Extensive measurement:** Can detect hormone potency, efficacy and receptor interaction.

Disadvantages

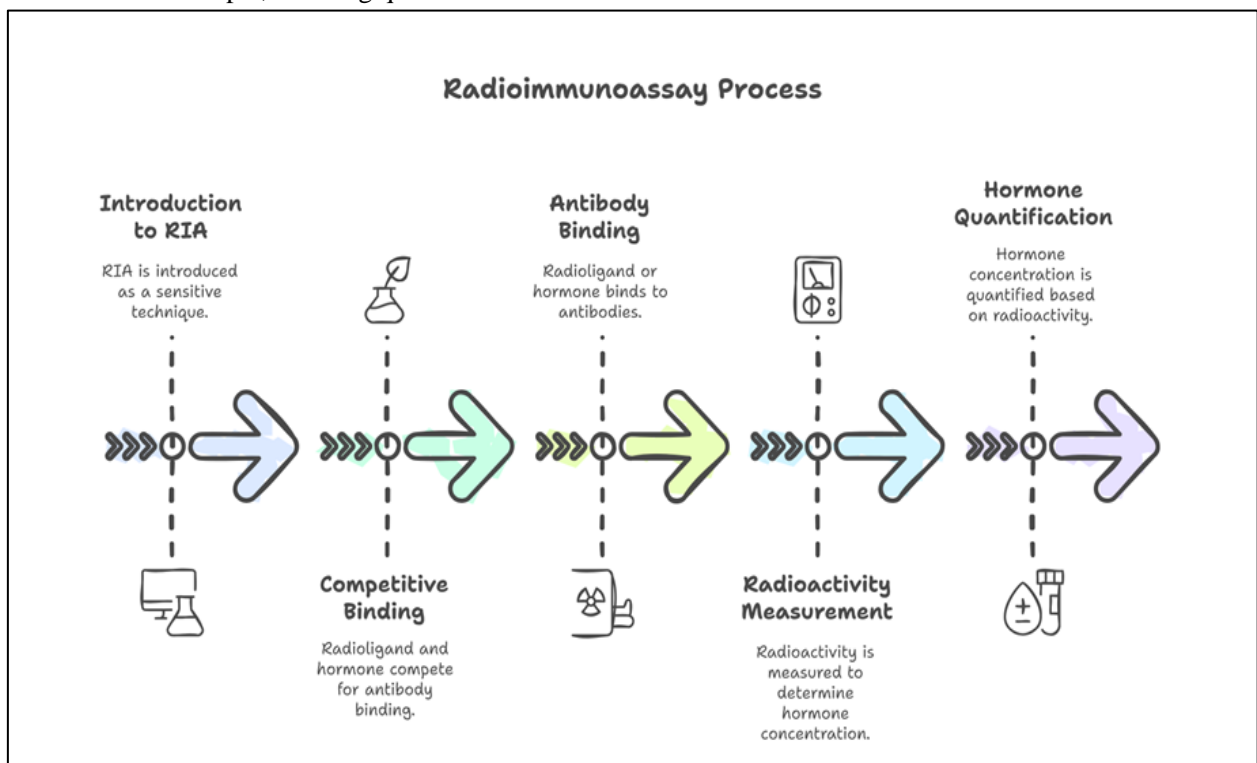
- **Low sensitivity and specificity:** Less sensitive compared to modern immunoassays and mass spectrometry, limiting detection of low hormone levels. Cross reactivity leads to low specificity.
- **Labor intensive and time-consuming:** Requires preparation of living tissues or organisms, which involves complex care and longer assay times.
- **Variability:** Biological systems may differ between assays, impacting reproducibility and reliability.
- Due to above technical challenges, bioassays are rarely used in routine clinical hormone testing today.

Radioimmunoassay (RIA):

RIA is a highly sensitive and specific laboratory technique developed in the 1960s to measure minute concentrations of hormones.

Principle

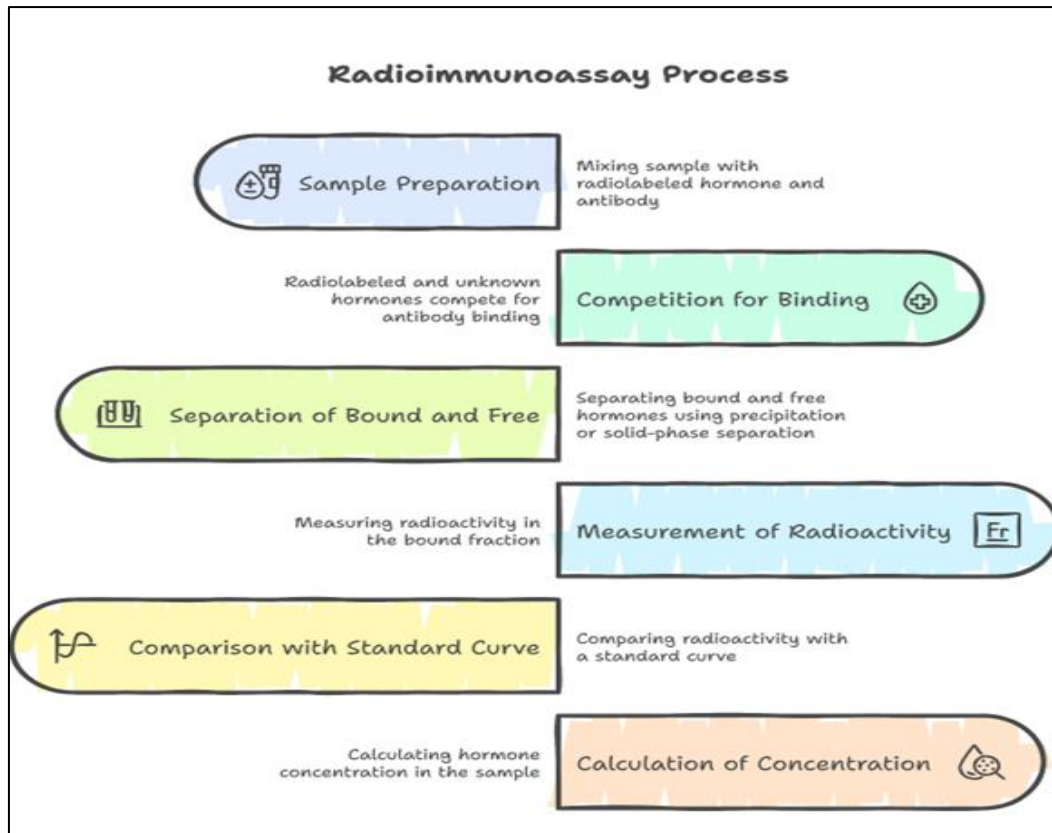
It is based on the principle of competitive binding between a radioactively labeled hormone (radio ligand) and the unlabeled hormone in the patient's sample for a limited number of specific antibody binding sites. The amount of radioactivity bound to the antibody is inversely proportional to the concentration of the hormone in the sample, allowing quantification.



Types of RIA

Competitive RIA: The patient's hormone competes with a known quantity of radiolabeled hormone for antibody binding. The concentration of hormone has an inverse relation with bound radioactivity.

Non-Competitive RIA (Sandwich Assay): involves capturing the hormone between two antibodies, one of which is labeled.



Advantages

- **High Sensitivity:** Can detect hormone concentration in picogram to nanogram range.
- **High Specificity:** reducing cross-reactivity by using hormone specific antibodies.
- **Multiple Application:** Used for a wide range of hormones including insulin, thyroid hormones, corticosteroids, and reproductive hormones.

Disadvantages

- **Radioactive Hazard:** radioactive isotopes are used in this technique which require special handling, disposal, and regulatory compliance.
- **Limited Shelf Life:** Radio labeled reagents have shorter life.
- **Technical Complexity:** Requires dedicated, trained personnel and specialized equipment.
- **Time-Consuming:** Multiple steps including separation and counting take longer than modern assays.

Clinical relevance of RIA currently

In clinical endocrinology, RIA was a breakthrough in diagnosing disorders like thyroid diseases, adrenal insufficiency, and reproductive abnormalities by enabling accurate hormone quantification, and it established

the foundation for modern hormone testing techniques. The development of safer, faster, and equally sensitive immunoassays like ELISA and chemiluminescence assays has reduced the clinical utilization of RIA for analysis of hormones. Use of RIA is limited only to certain specialized laboratories for hormones where other assays are unavailable or less validated.

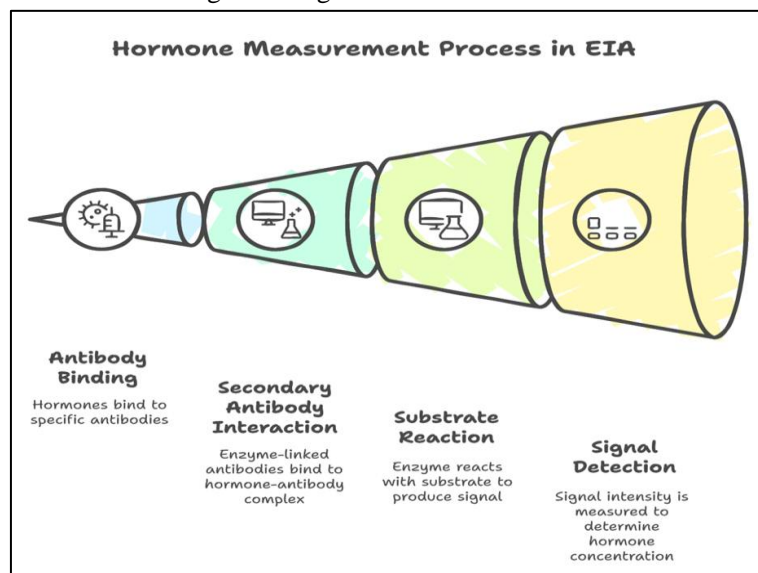
Modern analytical methods for hormone measurement

Hormone measurement technique had undergone significant evolution from conventional and radioimmunoassay to complex, more sensitive and more specific analytical techniques. Mass spectrometry, biosensor-based technologies are most widely used recent techniques which are integrated with advanced computational tools. Due to high sensitivity, specificity, and automation capabilities Immunoassays, such as enzyme-linked immunosorbent assays (ELISA) and chemiluminescence assays, have been the mainstay in the hormone analysis since decades. However, they sometimes face challenges with cross-reactivity and lower accuracy at very low hormone concentrations. Mass spectrometry, especially liquid chromatography-tandem mass spectrometry (LC-MS/MS), represents the gold standard for many steroid and peptide hormone assays. MS allows simultaneous measurement of multiple hormones with exceptional specificity and accuracy by directly detecting molecular structures. Despite technological complexity, it is increasingly adopted in clinical labs. Emerging biosensors and wearable technologies offer real-time and non-invasive hormone monitoring from biological fluids like saliva, sweat, or interstitial fluid, supported by AI-driven data analytics for personalized endocrinology. Recent techniques supporting and improving the clinical decision making with precise hormone measurement and reduce variability, making them indispensable tools in current endocrinology practice.

Enzyme Immunoassays (EIA):

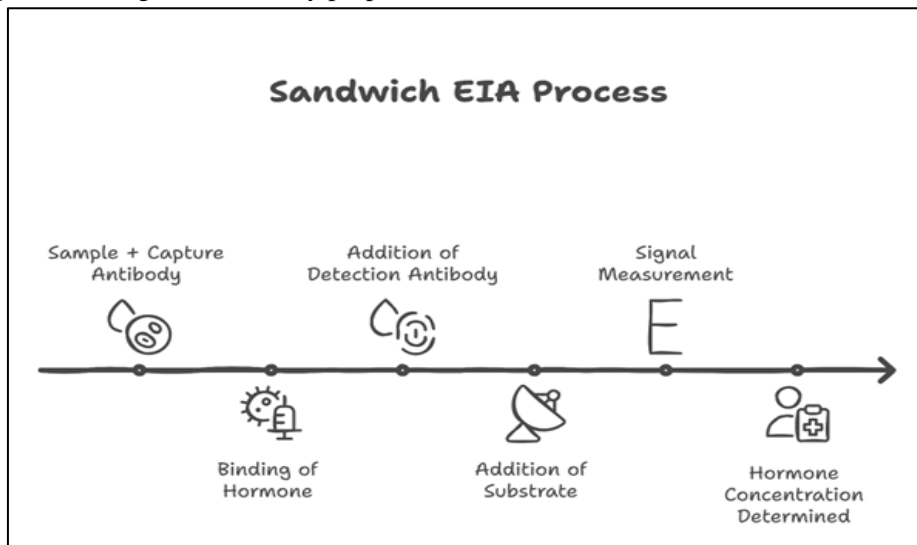
Principle

Enzyme immunoassays (EIA), including the widely used enzyme-linked immunosorbent assays (ELISA), are sensitive techniques that measure hormones based on antibody-antigen interactions. An enzyme-linked secondary antibody that acts on a substrate to produce a colorimetric, fluorescent, or luminescent signal is used to detect the hormone (antigen) in the sample, which binds to particular antibodies. The hormone concentration is correlated with the signal strength.



Types of Enzyme Immunoassays

- **Direct EIA:** The enzyme-labeled antibody binds directly to the hormone in the sample.
- **Indirect EIA:** The primary antibody binds to the hormone first, and then an enzyme-labeled secondary antibody binds to the primary antibody.
- **Sandwich EIA:** Uses two antibodies; one captures the hormone, and the second enzyme-linked antibody detects it, providing higher specificity.
- **Competitive EIA:** The hormone in the sample competes with an enzyme-labeled hormone for antibody binding sites; the signal is inversely proportional to the hormone concentration.



Advantages

- **High sensitivity and specificity:** Due to specific antigen antibody interaction and enzyme amplification.
- **Non-radioactive:** Safer compared to radioactive methods like RIA.
- **Quantitative and reproducible:** recommended for automated high-throughput lab testing.
- **Versatile:** Can measure a wide range of hormones and other biomolecules.
- **Relatively cost-effective and accessible:** Widely used in clinical and research labs.
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Disadvantages

- **Cross-reactivity:** Possible interference from structurally similar molecules may affect specificity.
- **Matrix effects:** Complex sample components (e.g., serum proteins) can interfere with the assay.
- **Limited multiplexing:** Usually designed for one hormone per assay.
- **Substrate and enzyme stability:** Assay conditions and reagent quality affect reliability.

Clinical relevance currently

Enzyme immunoassays are the cornerstone of most routine clinical hormone testing, including thyroid function tests, reproductive hormones, adrenal hormones, and metabolic regulators. They provide rapid, accurate results essential for diagnosis, treatment monitoring, and screening of endocrinological diseases.

EIA are now feasible and effective for clinical laboratories worldwide because of automated platforms with advanced computational tools.

Mass spectrometry

Despite of the technological complexity, Mass spectrometry, especially liquid chromatography-tandem mass spectrometry (LC-MS/MS), is the gold standard for analysis of multiple hormones greater degree of specificity and accuracy.

Liquid Chromatography-Mass Spectrometry (LC-MS): Comprehensive Overview

Principle

Liquid Chromatography-Mass Spectrometry (LC-MS) work principally on two powerful analytical techniques for hormone measurement. Liquid chromatography (LC) helps in separation of components of hormones on the basis of their chemical properties, while mass spectrometry (MS) has role in quantification of the hormones by measuring their mass-to-charge (m/z) ratios. The tandem mass spectrometry is more sensitive in detection of (closely related molecules).

Types of LC-MS

- **MALDI-TOF mass spectrometry:** Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a technique in which laser energy is used as ionization technique. It is used for biomolecules like carbohydrates and peptides.
- **Triple quadrupole mass spectrometry:** The triple quadrupole mass spectrometer (TQMS or QqQ), is a tandem mass spectrometer in which the first and third quadrupoles act as mass filters while the second acts as a collision cell which has role in fragmentation of the selected parent ions, a to generate daughter ions.
- **Quadrupole trap mass spectrometry:** A hybrid triple mass spectrometer in which third quadrupole can work as either a standard quadrupole mass filter or a linear ion trap (LIT). This will increase the sensitivity
- **Hybrid linear on trap orbitrap mass spectrometry:** combination of a linear ion trap and high-resolution Orbitrap widely used in proteomics and metabolomics analysis.
- **Quadrupole-Orbitrap mass spectrometry:** Combination of the high-performance quadrupole precursor selection with high-resolution, accurate-mass (HR/AM) Orbitrap detection.

Advantages

- **High specificity and sensitivity:** Differentiates hormones and metabolites with similar structures.
- **Multiplexing capability:** multiple hormones can be measured simultaneously in one run. Also suitable for complex matrix like serum, plasma and tissue extracts
- **Quantitative accuracy:** Minimal cross-reactivity and interference issues.
- **Wide applicability:** Effective for steroids, peptides, and other small molecules.

Disadvantages

- Expensive equipment and maintenance. So cannot be available in all clinical laboratories.

- Technically requires skilled personnel to operate and for sample preparation.
- Sample processing take longer time as compared to immunoassays.

Clinical Relevance Currently

LC-MS/MS is considered the gold standard for measuring steroid hormones (cortisol, aldosterone, and sex steroids) and peptide hormones where immunoassays have limitations. Its ability for multiplexing and high specificity is vital in diagnosing complex endocrine disorders and monitoring therapy. Despite cost and technical demands, its clinical use is expanding in reference and specialized labs, offering unparalleled accuracy in hormone measurement.

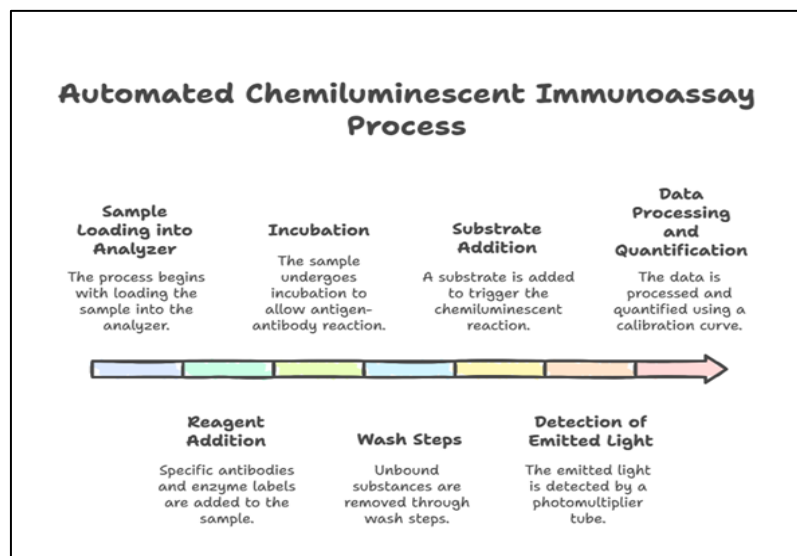
Automated Hormone Analyzers

Principle

Automated hormone analyzers are based on immunoassay principles—such as chemiluminescence, enzyme immunoassay, fluorescence immunoassay, or electrochemiluminescence. Automated immunoassay detects hormone antibody interactions accurately, rapidly with the help of advance detection technologies.

Types of Automated Hormone Analyzers

- **Chemiluminescent Immunoassay:** Detect light emitted from a chemiluminescent reaction triggered by enzyme-labeled antibodies.
- **Electrochemiluminescence (ECL):** Uses electrochemically stimulated light emission for highly sensitive detection.
- **Fluorescence Immunoassay Analyzers:** Detect fluorescent signals from labeled antibodies.
- **Enzyme-Linked Immunosorbent Assay (ELISA) Automated Systems:** Automate traditional ELISA steps for higher throughput.
- **Multiplex Analyzers:** Measure multiple hormones simultaneously using bead-based or array technologies.



Advantages

- **High throughput:** Processes large numbers of samples quickly.
- Automation helps in minimizing manual errors.
- **High sensitivity and specificity:** Uses advanced immunoassay formats.
- **Multiplexing:** Some systems measure several hormones simultaneously.
- Automated analyzers are easy to operate.

Disadvantages

- **High initial cost:** Expensive instruments and maintenance. Requires regular calibration and quality controls, which additionally increases the cost.
- Technical Expertise and skilled staff are required for operation and troubleshooting.
- Potential risk of Cross-Reactivity.

Clinical relevance currently

Though there is little limitation, still automated hormone analyzers based on immunoassay are the backbone of modern clinical laboratories. They enable rapid, precise hormone assessment essential for diagnosing thyroid diseases, adrenal and gonadal disorders, metabolic conditions, and growth abnormalities. They are useful for large-scale diagnosis and monitoring of patient condition, facilitating timely medical decision-making.

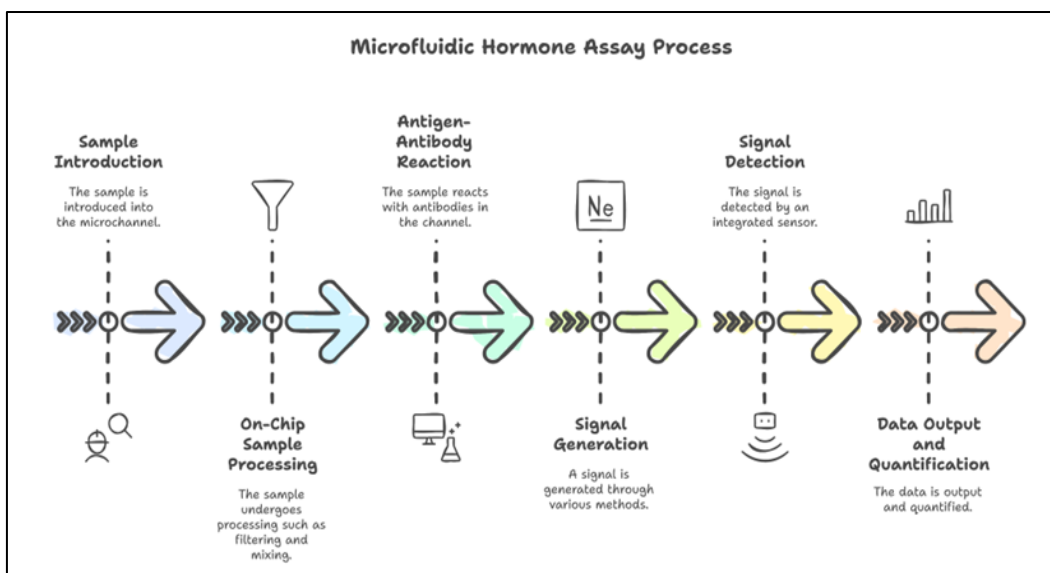
Micro fluidic devices for hormone measurement:

Principle

Microfluidic devices analyze molecules using very small volumes of fluids (microliters to picolitres) through microscale channels. Physical forces like capillary action, electro kinetics, or pressure are used to allow the entry of sample to these micro channels. For hormone measurement, these devices integrate sample processing, biochemical reactions (such as immunoassays), and detection on a single chip known as “lab-on-a-chip”. The main benefits of miniaturization are the handling of extremely small fluid volumes of reagents and samples, possibility of multiplexing the various operations, portability, disposability, low-cost, high throughput and low power consumption.

Types of microfluidic devices

- **Continuous flow microfluidics:** Fluid moves continuously through microchannels driven by pressure or pumps.
- **Droplet-based microfluidics:** Discrete droplets act as individual reaction vessels allowing precise control of biochemical reactions.
- **Paper-based microfluidics:** Utilizes patterned paper for fluid flow by capillary action; low-cost and portable.
- **Digital microfluidics:** Manipulates droplets on an array using electric fields, enabling programmable assays.



Advantages

- **Minimal sample and reagent volume:** permits testing with limited clinical samples.
- **Rapid turnaround:** Accelerated reaction kinetics due to small volumes.
- **Portability:** can be implemented as point-of-care (POC) and point of- need (PON) diagnostics that would enable quick and accurate results, leading to improved clinical outcome.
- **Integration:** Combines multiple laboratory procedures on a single chip.
- **High sensitivity:** Enhanced detection due to confined reaction environments.

Disadvantages

- **Complexity:** manufacturing techniques are complex and require specialized methods. Variable device designs lead to challenges in standardization.
- **Limited Throughput:** appropriate for single or small batch testing.
- **Sample Compatibility:** pretreatment is required for Complex biological fluids.
- **Cost:** Initial development and device cost may be high despite reagent savings.

Clinical Relevance Currently

To enable timely diagnosis and monitoring at bedside Microfluidic devices are increasingly explored for point-of-care hormone testing such as cortisol, insulin, and reproductive hormones. Their portability and speed offer advantages in emergency settings and in areas with limited resources. Although still emerging compared to conventional assays, microfluidic technologies have potential for personalized endocrinology and home-based testing applications.

Biosensors for hormone measurement

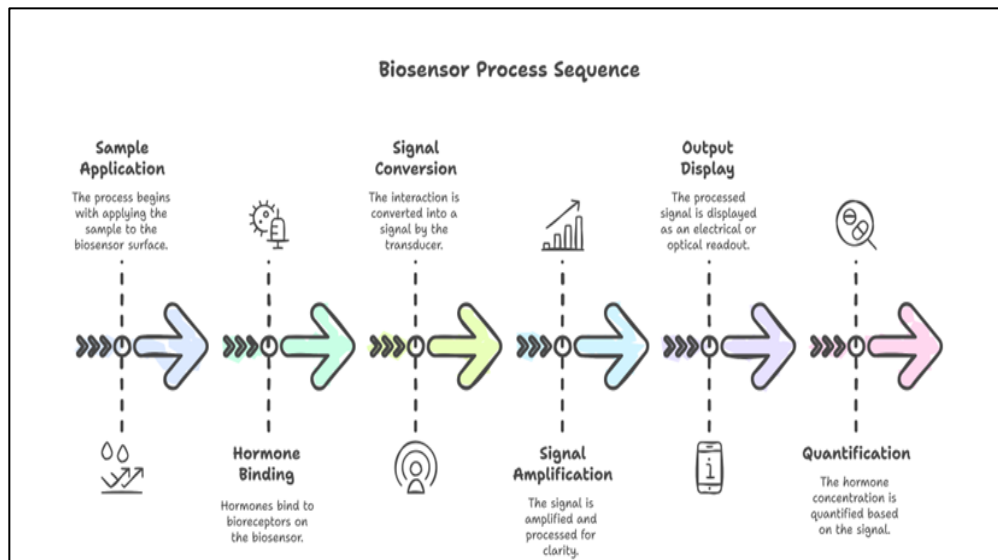
Principle

Biosensors are analytical devices that convert a biological reaction into a measurable signal. They consist of a bioreceptor (such as antibodies, enzymes, or nucleic acids) that specifically interacts with the hormone of

interest, integrated with a conversion unit that converts this interaction into an electrical, optical, or electrochemical signal. The concentration of hormone is then determined by quantifying the signal.

Types of Biosensors

- **Electrochemical biosensors:** Measure electrical signals caused by hormone-receptor interactions.
- **Optical biosensors:** Detect changes in light properties, including fluorescence, absorbance, or surface plasmon resonance.
- **Piezoelectric biosensors:** Sense changes in mass or acoustic waves upon hormone binding.
- **Thermal biosensors:** Measure heat changes generated during biochemical reactions.
- **Wearable biosensors:** Integrate conformable, wearable sensors for continuous monitoring.



Advantages

- **High sensitivity and specificity:** Bioreceptors ensure selective hormone detection.
- **Rapid turnaround time:** Enables near-instantaneous results.
- **Minimal Sample Volume.**
- **Portability:** Allows point-of-care and wearable applications.
- **Potential for continuous monitoring:** Particularly useful in chronic disease management.
- **Precise sensor technology.**

Disadvantages

- **Stability issues:** Bioreceptors may lose activity over time in adverse environment.
- **Challenges in calibration:** Requires standardization and frequent recalibration.
- **Interference:** Complex biological fluids can affect sensor performance.
- **Manufacturing complexity:** technically challenging to integrate biological and electronic components in a single device.
- **Cost:** High development and production costs can limit accessibility.

Clinical relevance currently

Biosensors represent a frontier in hormone measurement, specially for continuous or point-of-care monitoring of stress hormones (e.g., cortisol), reproductive hormones, and metabolic regulators. Wearable biosensors have received tremendous attention due to their potential to provide continuous and real time physiological information about health.

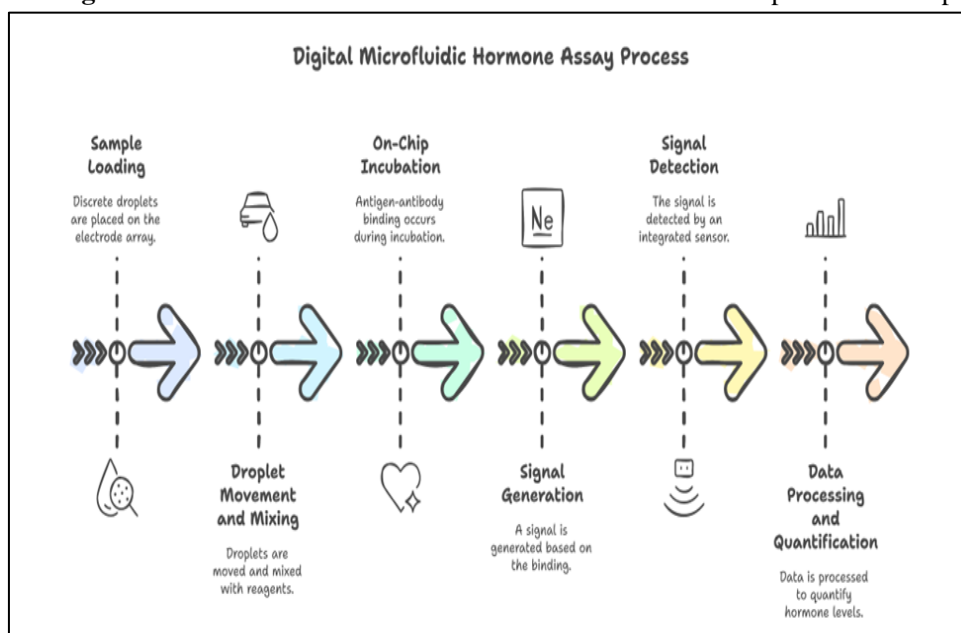
Digital microfluidics for hormone measurement

Principle

Digital microfluidics (DMF) uses electrical forces, typically electrowetting-on-dielectric (EWOD) to manage the discrete fluid droplets on a planar surface. By applying voltages to electrodes beneath an insulating layer, droplets can be precisely moved, merged, split, mixed, or dispensed on the chip. This enables automated, programmable biochemical reactions, including hormone immunoassays, using minimal sample and reagent volumes.

Types of Digital Microfluidics

- **Electrowetting-on-Dielectric (EWOD):** The most common DMF method controlling droplets via changes in surface tension induced by electric fields.
- **Dielectrophoresis (DEP):** Uses non-uniform electric fields to manipulate droplets via induced dipoles.
- **Magnetic Digital Microfluidics:** Manipulates droplets containing magnetic particles via external magnetic fields.
- **Acoustic Digital Microfluidics:** Uses acoustic waves to move or mix droplets on the chip.



Advantages

- **Programmable and flexible:** Automated control over assay steps on a single device.
- **Minimal reagents and sample:** Reduces consumption and cost.
- **Rapid reaction times:** Efficient mixing in picolitre to nanoliter droplets.

- **Integrated and portable:** Potential for point-of-care testing and multiple assay steps can be combined.
- Disadvantages**

- **Fabrication complexity:** Requires advanced microfabrication technology for manufacturing.
- **Limited throughput:** Typically suited for single or low sample volumes compared to continuous flow systems.
- **Cost and expertise:** High development cost and need for specialized operation knowledge.
- **Sample compatibility:** May need preprocessing for complex biological fluids. Small sample are susceptible to evaporation.

Clinical relevance currently

Digital microfluidics holds promise for point-of-care, rapid hormone testing with automation and minimal sample use. It facilitates personalized and decentralized healthcare by enabling complex assays outside centralized labs. Though still largely in research and early clinical use phases, DMF technology is anticipated to expand in hormone diagnostics due to its flexibility, speed, and integration capabilities.

Clinical application: Endocrine disorder evaluation, reproductive Health Monitoring, tumor markers, biochemical screening for antenatal screening, Therapeutic Drug Monitoring- Steroid Drug Level Tracking, Personalized Medicine Approaches, Sports Anti-doping Analysis- Banned Substance Screening, Longitudinal Athlete Profiling.

Conclusion

Advancements in hormone analysis have been propelled by various technologies. Enzyme immunoassays offer enhanced sensitivity but can have interferences. Mass spectrometry, particularly liquid chromatography–tandem mass spectrometry (LC-MS/MS), offers accuracy and specificity but requires complex instrumentation. Microfluidics is portable and can be used as POCT device but have limited throughput. Biosensors, including wearable types, provide rapid, non-invasive hormone detection but struggle with stability and integration. The continued integration and advancement of these technologies promise improved disease management and patient outcomes in endocrine diagnostics.

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