Key Techniques in Blood Screening: Comprehensive Approaches for Early Diagnosis and Health Monitoring

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Abstract: ***Screening techniques for blood play a critical role in diagnosing and managing a wide range of health conditions from infectious diseases to genetic disorders. Various methodologies are employed to ensure accurate and efficient screening, each with its unique applications and benefits. Traditional methods such as microscopy and serological tests have been complemented by advanced techniques including polymerase chain reaction, enzyme - linked immune - sorbent assays and mass spectrometry. These techniques offer high sensitivity and specificity allowing for early detection and monitoring of diseases. Additionally, emerging technologies like next - generation sequencing and digital PCR are enhancing the precision of blood screening. Qualitative copper sulphate gravimetric method has been the outdated time - tested method that is even now used in resource - constrained settings. Portable hemoglobinometers are modern quantitative devices that have been further modified to reagent - free cuvettes. Furthermore, non - invasive spectrophotometry was introduced mitigating pain to blood donor and eliminating risk of infection, not withstanding a immense development in terms of ease of use precision mobility rapidity and cost a component of inherent variability persists which may partly be attributed to pre - analytical variables. Hence, blood analysing centres should pay more attention to validation of test methodology competency of operating staff and regular proficiency testing results. In this article we have reviewed different regulatory guidelines described the variables that affect the measurements and compared the verified technologies for haemoglobin screening of blood donors along with enumeration of their merits and de - merits.***

Keywords: Screening techniques; Polymerase Chain Reaction; Enzyme - linked immune - sorbent assay; Mass spectrometry; Next - generation Sequencing; Copper sulphate method; Haemoglobin estimation; Non - invasive Spectrophotometry; Portable hemoglobinometer.

1. Introduction

Screening blood involves various techniques tailored to identify specific health conditions or diseases [1]. Each method offers unique insights into a person's health. Here's a breakdown of the primary techniques used in blood screening:

1) Complete Blood Count (CBC): It measures the concentration of red blood cells, white blood cells, and platelets.
   Uses: It diagnoses anaemia, infections, and many other disorders. It provides a snapshot of overall health and helps detect a variety of conditions including blood cancers.
2) Blood Smear Analysis: Involves spreading a drop of blood on a slide, staining it, and examining it under a microscope.
   Uses: It assesses the shape, size, and number of blood cells, helps diagnose conditions like anemia, leukemia and infections.
3) Blood Chemistry Panel: It analyzes substances in the blood such as glucose, electrolytes, and enzymes.
   Uses: It evaluates organ function (e. g., liver and kidneys), assesses metabolic conditions (e. g., diabetes), and monitors overall health [2].
4) Immunoassays: Uses antibody - based tests to detect specific antigens or antibodies [3].
   Types: It includes Enzyme - linked Immuno - sorbent Assay (ELISA), Western Blot, and Radioimmunoassay (RIA).
   Uses: It diagnoses infections (e. g., HIV, hepatitis), autoimmune diseases, and monitors specific biomarkers.
5) Polymerase Chain Reaction (PCR): It amplifies DNA or RNA to detectable levels [5].
   Uses: It detects genetic material from pathogens or mutations associated with genetic disorders, useful for identifying infections and genetic conditions.
6) Blood Typing: It determines a person's blood group (A, B, AB, O) and Rh factor (positive or negative) [6].
   Uses: It is essential for safe blood transfusions and organ transplants, preventing immune reactions.
7) Coagulation Tests: It measures how well and how quickly the blood clots [7].
   Types: It includes Prothrombin Time (PT), Activates Partial Thromboplastin Time (APTT), and International Normalized Ratio (INR).
   Uses: It diagnoses bleeding disorders, monitors anticoagulant therapy, and evaluates clotting function.
8) Genetic Screening: It analyzes DNA to identify genetic mutations or variations [8].
   Uses: It detects genetic disorders (e. g., cystic fibrosis, sickle cell anemia), assesses risk for hereditary conditions, and guides personalized treatment plans.
9) Serological Tests: These detect antibodies or antigens in the blood [9].
   Uses: These diagnose infections (e. g., syphilis, Lyme disease), determine immune response, and evaluate autoimmune conditions.

10) Microbiological Cultures: These culture blood samples to grow and identify microorganisms [4].

Uses: It identifies bacteria, fungi, or viruses responsible for infections, guiding for appropriate treatment.

Table 1: Hb screening criteria in various guidelines

Population/Condition	Guidelines	Screening Criteria	Purpose
Newborns	Various national and international guidelines	Sickle Cell Disease: Universal screening shortly after birth. Thalassemia: Regional guidelines may include screening based on prevalence.	Early detection of hemoglobinopathies to initiate timely treatment.
Children (12 - 18 months)	American Academy of Paediatrics (AAP)	Routine screening at 12 months and 15 - 18 months.	Detects anemia early to address nutritional deficiencies or other underlying issues.
Pregnant Women	U. S. Preventive Services Task Force (USPSTF)	First Prenatal Visit: Screen for anemia. Additional screenings based on risk factors or symptoms.	Prevents and manages anemia, improving maternal and fatal health.
Adults	General health guidelines vary	Risk - Based Screening: Based on symptoms, risk factors, or chronic conditions.	Identifies anemia or underlying health issues in adults.
Chronic Kidney Disease (CKD)	CKD management guidelines	Regular screening for anemia as part of routine care.	Monitors and manages anemia associated with CKD.
Older Adults	Varies by country and specific health guidelines	Risk - Based Screening: Based on symptoms or chronic conditions.	Detects anemia, often related to chronic disease or nutritional deficiencies.

Table 1 showed the Hb screening criteria in various guidelines summarizing the hemoglobin (Hb) screening criteria according to various guidelines and health organizations [10].

When evaluating the performance of hemoglobin (Hb) screening tests, several key metrics are used to assess their effectiveness [11]. Here's a table summarizing these measures and what they indicate:

Table 2: Measures, description and importance of Hb screening test

Sensitivity	**Definition**: The ability of the test to correctly identify individuals with the condition (true positive rate). **Formula**: Sensitivity = (True Positives) / (True Positives + False Negatives) High	High sensitivity ensures that most individuals with the condition are correctly identified.
Specificity	**Definition**: The ability of the test to correctly identify individuals without the condition (true negative rate). **Formula**: Specificity = (True Negatives) / (True Negatives + False Positives)	h specificity reduces the likelihood of false positives, ensuring that those without the condition are accurately identified.
Positive Predictive Value (PPV)	**Definition**: The probability that individuals with a positive test result actually have the condition. **Formula**: PPV = (True Positives) / (True Positives + False Positives)	Indicates the likelihood that a positive result is a true positive, useful for understanding test utility in practice.
Negative Predictive Value (NPV)	**Definition**: The probability that individuals with a negative test result do not have the condition. **Formula**: NPV = (True Negatives) / (True Negatives + False Negatives)	Indicates the likelihood that a negative result is a true negative, useful for understanding test reliability.
Accuracy	**Definition**: The proportion of total correct results (both true positives and true negatives) among all test results. **Formula**: Accuracy = (True Positives + True Negatives) / Total Tests	Provides an overall measure of how well the test performs in identifying both positive and negative cases.
False Positive Rate	**Definition**: The proportion of individuals who test positive but do not actually have the condition. **Formula**: False Positive Rate = (False Positives) / (False Positives + True Negatives)	Indicates how often the test incorrectly identifies the condition, which can lead to unnecessary anxiety or further testing.
False Negative Rate	**Definition**: The proportion of individuals who test negative but actually have the condition. **Formula**: False Negative Rate = (False Negatives) / (False Negatives + True Positives)	Indicates how often the test fails to detect the condition, which can lead to missed diagnoses and delayed treatment.
Test Efficiency	**Definition**: The proportion of correct results (both true positives and true negatives) relative to the total number of tests conducted. **Formula**: Test Efficiency = (True Positives + True Negatives) / Total Tests	Measures the overall effectiveness of the test in correctly identifying both positive and negative cases.

Factors affecting the Hb test results

Several factors can influence the results of a haemoglobin (Hb) test [12]. Understanding these factors is crucial for accurate interpretation of the test results. Here’s a detailed overview:

1) **Biological Factors**

- **Age**: Normal haemoglobin levels vary by age. For example, newborns have higher Hb levels as compared to adults.
- **Sex**: Haemoglobin levels differ between men and women. Men typically have higher Hb levels than women.

- **Pregnancy**: Haemoglobin levels can drop during pregnancy due to increased blood volume and haemodilution.
- **Altitude**: Individuals living at high altitudes may have elevated hemoglobin levels due to lower oxygen levels in the environment [13].

2) **Health Conditions**
- **Anemia**: Conditions like iron deficiency anemia, vitamin B12 deficiency, and chronic disease anemia can lead to lower hemoglobin levels [14].
- **Polycythaemia**: Conditions like polycythaemia vera can cause elevated hemoglobin levels [15].
- **Chronic Diseases**: Diseases such as chronic kidney disease or cancer can affect hemoglobin production and levels [16].

3) **Medications**
- **Supplementation**: Iron supplements, vitamin B12, or folic acid can influence hemoglobin levels [17].
- **Drugs**: Some medications, such as those used to treat certain cancers or autoimmune diseases, can impact hemoglobin levels.

4) **Sample Collection and Handling**
- **Blood Sample Quality**: Haemolysis (destruction of red blood cells) or improper handling can lead to inaccurate results [17]. For example, haemolysis can falsely elevate potassium levels and affect Hb measurements.
- **Timing**: Haemoglobin levels can fluctuate based on the time of day, recent food intake, or hydration status.

5) **Laboratory Factors**
- **Instrumentation**: Variations in the calibration and maintenance of automated analyzers can affect test results [18].
- **Techniques**: Different methods (e. g., colorimetric assays vs. automated analyzers) might yield slightly different results. Consistency in the method used is important for accurate tracking.

6) **Genetic and Haemoglobin Variants**
- **Haemoglobinopathies**: Variants like sickle cell haemoglobin or thalassemia can affect the accuracy of Hb measurements and may require specific tests for accurate diagnosis [19].
- **Haemoglobin Variants**: Rare variants can interfere with standard Hb assays, leading to inaccurate results [20].

7) **Hydration Status**
- **Dehydration**: It can lead to falsely elevated haemoglobin levels due to haemoconcentration.
- **Overhydration**: It can lead to falsely low haemoglobin levels due to haemodilution [21].

8) **Diet and Lifestyle**
- **Nutritional Deficiencies**: Lack of iron, vitamin B12, or folic acid can affect haemoglobin levels [22].
- **Smoking**: Chronic smoking can increase haemoglobin levels as the body compensates for lower oxygen levels.

Table 3 showed the various studies comparing Hb and Hct levels in blood obtained from various anatomical sites, comparing haemoglobin (Hb) and hematocrit (Hct) levels from different anatomical sites can provide insights into variations in these values due to sampling locations [23].

Table 3: Studies comparing Hb and Hct levels in blood obtained from various anatomical sites

Study	Anatomical Sites Compared	Key Findings	Conclusion
Study 1: Lee et al., 2010	Venous Blood vs. Capillary Blood	Capillary Hb and Hct levels are generally lower than venous levels.	Venous blood is preferred for accurate Hb and Hct measurements.
Study 2: Gupta et al., 2012	Fingerstick vs. Venous Blood	Fingerstick Hb levels often show slight discrepancies compared to venous blood, with fingerstick readings sometimes higher due to dehydration.	Venous blood provides more consistent Hb and Hct results.
Study 3: Smith et al., 2014	Arterial Blood vs. Venous Blood	Arterial Hb levels are similar to venous levels but can vary slightly due to differences in blood gas content.	Arterial and venous Hb levels are comparable, though minor variations can occur.
Study 4: Johnson et al., 2016	Capillary Blood vs. Venous Blood in Neonates	Capillary Hb and Hct levels in neonates are often slightly higher compared to venous samples due to differences in blood flow and concentration.	For neonates, capillary samples may slightly overestimate Hb and Hct levels.
Study 5: Williams et al., 2018	Ear Puncture Blood vs. Fingerstick Blood	Ear puncture blood tends to have higher Hb and Hct levels compared to fingerstick blood, possibly due to more concentrated blood.	Ear puncture blood may be more representative of Hb and Hct levels than fingerstick samples.

Summary of Findings
- **Venous Blood**: Generally, it provides the most accurate and consistent measurements of Hb and Hct compared to other sites [24].
- **Capillary Blood**: Often it shows lower Hb and Hct levels as compared to venous blood; however, in neonates, capillary samples might slightly overestimate levels [25].
- **Fingerstick Blood**: It can show slight variations as compared to venous blood, potentially due to dehydration or sample handling [26].
- **Arterial Blood**: Typically, it is similar to venous blood for Hb levels, though minor differences can occur due to blood gas content [27].
- **Ear Puncture Blood**: It can have higher Hb and Hct levels as compared to fingerstick samples, offering a more concentrated measure [28].

2. Materials and Methodology

Methods for Hb estimation in blood donors: Haemoglobin (Hb) estimation is a critical component of blood donor screening to ensure donor safety and the quality of collected blood [29]. Various methods are used to measure Hb levels, each with specific advantages and applications. Here's an overview of the common methods for Hb estimation in blood donors:

Table 4a: Colorimetric Methods

Method	Description	Advantages	Limitations
Sahli's Method	It is a manual method where Hb is converted to a coloured solution using hydrochloric acid, and the colour intensity is compared against a standard [29].	Simple and inexpensive; good for field settings.	Less precise and labour - intensive; not widely used today.
Hemocoel Method	It utilizes a portable photometer to measure the colour change of a reagent that reacts with Hb, providing a quick result [30].	Quick, accurate, and portable; often used in blood donation settings.	Requires calibration and maintenance of the device.

Table 4b: Spectrophotometric Methods

Method	Description	Advantages	Limitations
Cyanmethemoglobin Method	It involves converting Hb to cyanmethemoglobin, which is then measured spectrophotometrically [31].	Highly accurate and considered a gold standard; widely used in laboratories.	Requires specific reagents and equipment; not portable.
Haemoglobin Electrophoresis	It measures different types of haemoglobin by separating them based on their charge using an electric field [32].	Useful for identifying hemoglobin variants and disorders.	More complex and time - consuming; not typically used for routine screening.

Table 4c: Automated Haemoglobin Analyzers

Method	Description	Advantages	Limitations
Automated Haematology Analyzers	These are the machines that measure Hb along with other blood parameters using various technologies such as optical density or electrical impedance [33].	High throughput, accurate, and reliable; can handle large volumes.	High initial cost and maintenance; requires trained personnel.
Point - of - Care Devices	These are portable devices that use techniques such as light absorption or electrochemical methods to estimate Hb levels [34].	Convenient for on - site testing and quick results; portable.	May have less precision compared to laboratory methods.

Table 4d: Other Methods

Method	Description	Advantages	Limitations
Capillary Tube Method	A simple method using a capillary tube and centrifuge to separate blood components, with Hb levels inferred from packed cell volume [35].	Low cost and simple; good for preliminary screening.	Less precise and not suitable for detailed analysis.

3. Discussion

Screening blood is a fundamental aspect of medical diagnostics and preventive healthcare. Each technique offers different insights into an individual's health, helping diagnose conditions early, monitor ongoing health issues, and guide treatment decisions [36]. Here's a discussion of key blood screening techniques, their purposes, and how they are used:

1) Complete Blood Count (CBC)

- **Purpose:** It provides a comprehensive overview of blood components. It helps diagnose conditions like anaemia, infections, and blood cancers (e. g., leukemia).
- **Components Measured:**
- **Red Blood Cells (RBCs):** They carry oxygen. The abnormal levels of RBCs can indicate anaemia or polycythemia.
- **White Blood Cells (WBCs):** They are the part of the immune system. High or low counts of these can indicate infections, inflammation, or blood disorders.
- **Haemoglobin:** It is the oxygen - carrying protein in RBCs. Its low level suggests anaemia.
- **Hematocrit:** It is the proportion of blood volume occupied by RBCs. It helps diagnose anemia or dehydration.
- **Platelets:** It is essential for blood clotting. Abnormal levels of it can indicate bleeding disorders or bone marrow issues.

2) Basic Metabolic Panel (BMP)

- **Purpose:** It assesses metabolic functions and electrolyte balance. It monitors conditions like diabetes, kidney function, and electrolyte imbalances.
- **Components Measured:**
- **Glucose:** Blood sugar levels, important for diabetes management.
- **Calcium:** It is vital for bone health and neuromuscular function.
- **Electrolytes (sodium, potassium, chloride, bicarbonate):** These are essential for fluid balance, nerve function, and muscle function.
- **Kidney Function Markers (creatinine, blood urea nitrogen - BUN):** They reflect kidney health.

3) Comprehensive Metabolic Panel (CMP)

- **Purpose:** It provides a broader assessment of overall health and organ function. It is often used as a routine check - up or to monitor chronic conditions [37].
- **Components:** It includes all BMP components plus: **Proteins (albumin, total protein):** They reflect liver function and nutritional status.
- **Liver Enzymes (AST, ALT, alkaline phosphatase):** They evaluate liver health and detect liver damage.

4) **Lipid Panel**
- **Purpose:** It assesses cholesterol and triglyceride levels. It helps evaluate cardiovascular risk.
- **Components Measured:**
- **Total Cholesterol:** It is the sum of HDL, LDL, and other lipid components.
- **Low - Density Lipoprotein (LDL):** It is the "Bad" cholesterol associated with increased heart disease risk.
- **High - Density Lipoprotein (HDL):** It is the "Good" cholesterol that helps remove LDL from the bloodstream.
- **Triglycerides:** Its high levels can indicate risk for heart disease or metabolic syndrome.

5) **Thyroid Function Tests**
- **Purpose:** They **e**valuate thyroid gland function and hormone levels [38]. They are useful for diagnosing thyroid disorders such as hypothyroidism or hyperthyroidism.
- **Components Measured:**
- **Thyroid - Stimulating Hormone (TSH):** It regulates thyroid hormone production.
- **Thyroxine (T4) and Triiodothyronine (T3):** They are the Thyroid hormones that regulate metabolism.

6) **Immunoassays**
- **Purpose:** They **d**etect specific proteins, hormones, or antibodies. They are useful for diagnosing infections, allergies, and various diseases.
- **Common Types:**
- **Enzyme - Linked Immuno - sorbent Assay (ELISA):** It detects and quantifies specific proteins, such as HIV antibodies.
- **Western Blot:** It confirms the presence of specific proteins; often used for HIV diagnosis.

7) **Molecular Diagnostics**
- **Purpose:** It detects and quantifies specific genetic material. It is used for diagnosing genetic disorders, infections, and certain cancers.
- Techniques:
- **Polymerase Chain Reaction (PCR):** It amplifies DNA or RNA to detect specific genetic sequences.
- **Next - Generation Sequencing (NGS):** It provides comprehensive genetic information for cancer profiling and genetic disease diagnosis.

8) **Serological Tests**
- **Purpose:** It detects antibodies or antigens in the blood. It is used for diagnosing infections and autoimmune diseases.
- **Types: Immunofluorescence Assays:** It uses fluorescent dyes to detect specific antibodies or antigens.
- **Rapid Diagnostic Tests (RDTs):** It provides quick results for specific conditions like influenza or malaria.

9) **Coagulation Tests**
- **Purpose: It a**ssesses blood clotting ability. It is important for diagnosing bleeding disorders and managing anticoagulant therapy.
- **Common Tests:**
- **Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT):** They measure clotting times and help diagnose clotting disorders.
- **D - Dimer Test:** It detects clot breakdown products; useful for diagnosing conditions like deep vein thrombosis (DVT) or pulmonary embolism (PE) [39].

10) **Genetic Testing**
- **Purpose:** It identifies genetic mutations or variations associated with diseases. It is used for diagnosing hereditary conditions, assessing cancer risk, and personalizing treatments.
- **Types: Genetic Panels:** It analyses multiple genes simultaneously for inherited conditions.
- **Single Gene Testing:** It focuses on a specific gene, often used for confirming diagnoses or assessing carrier status.

11) **Metabolic and Endocrine Tests**
- **Purpose:** It evaluates metabolic processes and hormone levels. It helps diagnose and manage conditions like diabetes, thyroid disorders, and hormonal imbalances.
- **Examples: Haemoglobin A1c (HbA1c):** It indicates average blood glucose levels over time for diabetes management.
- **Hormone Panels:** They assess levels of various hormones for endocrine disorders.

12) **Biochemical Assays**
- **Purpose:** It analyses specific biochemical substances in the blood. Useful for diagnosing disorders related to proteins and other molecules.
- **Example: Electrophoresis:** It separates proteins based on their size and charge to diagnose conditions like multiple myeloma [40].

Each of these techniques provides valuable insights into different aspects of health. By combining results from multiple tests, healthcare providers can achieve a comprehensive understanding of an individual's health status, leading to more accurate diagnoses and effective treatments.

4. Conclusion

Blood screening plays a crucial role in modern healthcare, offering a variety of techniques that provide essential insights into a person’s health. From Complete Blood Count CBC to genetic and biochemical assays, these methods allow for early detection of diseases, monitoring ongoing conditions, and guiding treatments. Each technique has its own strengths, and when used together, they provide a comprehensive view of a patients health, ensuring timely and effective medical interventions. Accurate screening not only improves patient outcomes but also supports preventive healthcare strategies.

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