ISSN No:-2456-2165

# Comprehensive Study on Non-Invasive Blood Typing Techniques

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**Abstract:- Blood group detection, also known as blood typing or blood grouping, is of paramount importance in medical and healthcare settings. Blood transfusions are common in medical practice for patients who require blood due to surgeries, trauma, hemorrhage, or medical conditions like anemia. Blood groups are classified into different types, including A, B, AB, and O, as well as Rh-positive or Rh-negative. If incompatible blood is transfused, it can cause severe transfusion reactions, including hemolytic reactions, which can be life-threatening. Blood grouping helps to identify the correct blood type for transfusion, ensuring that the blood received by the patient is compatible and safe. It is critical in organ and tissue transplantation to ensure compatibility between the donor and recipient. For example, in kidney transplantation, blood group matching is necessary to prevent organ rejection and maximize the success of the transplant. It also plays a role in bone marrow transplantation, as the donor and recipient must have matching blood groups to reduce the risk of rejection and complications. It is vital during pregnancy and neonatal care to identify potential risks related to blood group incompatibility between the mother and the fetus. It helps to identify such risks and take appropriate preventive measures, such as administering Rh immune globulin, to protect the health of the mother and baby. It is also used in forensic medicine for forensic investigations and crime scene analysis. Blood stains and samples collected from crime scenes can be tested to determine the blood group of the individual involved, which can provide valuable information for criminal investigations and legal proceedings. Quick and accurate blood group detection can help ensure that the right blood type is administered promptly, reducing the risk of complications and improving patient outcomes. It plays a critical role in preventing adverse reactions, optimizing patient care, and ensuring the safety and well-being of patients in various medical scenarios.**

***Keywords:*** *Blood Groups, Antigen, Non-Invasive, NIR, LED.*

## I. INTRODUCTION

Blood grouping, also known as blood typing, is a laboratory test that determines the specific type of blood a person has based on the presence or absence of certain antigens (proteins) on the surface of red blood cells. The most commonly used blood grouping system is the ABO system, which categorizes blood into four main types: A, B, AB, and O. Another important blood grouping system is the Rh system, which classifies blood as Rh-positive (Rh+) or Rh negative (Rh-), based on the presence or absence of the Rh antigen. Other blood grouping systems, such as the Kell, Duffy, and Kidd systems, are also used in certain situations.

The standard method of blood grouping involves a series of tests using specific reagents to determine the presence or absence of antigens on the surface of red blood cells.

➢ *The Standard Method for Blood Grouping Typically Involves the Following Steps:*

- *Sample Collection*

A small blood sample is collected from the individual usually by venipuncture, where a needle is used to draw blood from a vein, or by fingerstick, where a small prick is made on the fingertip to collect a few drops of blood.

- *Preparation of Reagents*

Specific reagents, known as antisera, are prepared for each blood group being tested. Antisera are solutions containing antibodies that are designed to react with the antigens on the red blood cells.

- *Testing for ABO Blood Group*

The blood sample is mixed with antisera against A and B antigens separately. If the red blood cells agglutinate (clump) when mixed with anti-A serum, the blood is classified as blood group A. If agglutination occurs with anti-B serum, the blood is classified as blood group B. If agglutination occurs with both anti-A and anti-B sera, the blood is classified as blood group AB. If no agglutination occurs with either anti-A or anti-B serum, the blood is classified as blood group O.

- *Testing For Rh Blood Group*

Once the ABO blood group is determined, the blood sample is tested for Rh group. If agglutination occurs when the blood sample is mixed with anti-Rh serum, the blood is classified as Rh-positive (Rh+). If no agglutination occurs, the blood is classified as Rh-negative (Rh-).

- *Confirmation Testing*

To ensure accuracy, blood grouping results are typically confirmed by repeating the tests with additional reagents, and in some cases, by using additional methods such as forward and reverse typing.

- *Result Interpretation*

Based on the presence or absence of agglutination in reaction with different antisera, the blood type is determined.

The most commonly used tests for blood grouping are based on the ABO and Rh blood group systems, as these are the most clinically significant blood groups and have important implications for blood transfusion and organ transplantation. The following are the commonly used tests for blood grouping:

✓ *ABO Blood Grouping*

A series of tests are performed to determine an individual's ABO blood group. This typically involves testing the blood sample with anti-A and anti-B sera, which are reagents containing antibodies against A and B antigens, respectively. The reaction of the blood sample with these antisera determines the presence or absence of A and B antigens on the red blood cells, and hence the blood group is identified as A, B, AB, or O.

✓ *Rh Blood Grouping*

The Rh blood group system classifies blood as Rh-positive (Rh+) or Rh-negative (Rh-). This test involves testing the blood sample with anti-Rh sera, which are reagents containing antibodies against Rh antigens. If agglutination occurs, it indicates the presence of Rh antigens on the red blood cells, and the blood is identified as Rh+. If no agglutination occurs, it indicates the absence of Rh antigens, and the blood is identified as Rh-.

✓ *Antibody Screening*

This test involves testing the blood sample with a panel of screening cells that are known to express a variety of different antigens. The purpose of antibody screening is to detect the presence of unexpected antibodies in the blood, which could potentially cause transfusion reactions. If any unexpected antibodies are detected, further testing is performed to identify the specificity of the antibodies.

✓ *Cross Matching*

This is performed to determine the compatibility of blood for transfusion. It involves mixing the recipient's blood sample with the donor's blood sample, and checking for any agglutination or other reactions that may indicate incompatibility. It is typically done after ABO and Rh blood grouping and antibody screening, to ensure that the donor blood is compatible with the recipient's blood, and to minimize the risk of transfusion reactions.

➢ *Present Methods*

The most common methods used for blood group detection include:

- *Blood Group Serology*

This method involves using specific antibodies to detect the presence of antigens on the surface of red blood cells. Reagents containing known antibodies against A, B, and Rh antigens are mixed with the patient's blood sample, and the reactions are observed. Agglutination (clumping) of red blood cells indicates the presence of specific antigens, helping to determine the blood group type. This method is widely used in blood banks and clinical laboratories and is considered the gold standard for blood group detection.

- *Hemagglutination*

This method uses commercially available blood typing reagents that contain antibodies against A, B, and Rh antigens. The patient's blood sample is mixed with these reagents, and agglutination or lack of agglutination is observed, indicating the blood group type.

- *Polymerase Chain Reaction (PCR)*

PCR-based methods involve amplifying the DNA of the ABO and Rh genes from the patient's blood sample and then detecting specific gene sequences to determine the blood group type.

- *Automated Blood Group Analyzers*

These are automated systems that use various techniques, such as microplate agglutination, solid-phase technology, or gel card methods, to detect blood group antigens and antibodies. These systems offer high throughput and efficiency, with automated interpretation and reporting of results.

- *Point-of-Care Testing (POCT)*

Some rapid blood group detection methods are available as POCT, which can provide quick results at the patient's bedside or in remote settings. These may include lateral flow immunoassays or other rapid testing methods, which can provide preliminary blood group information for urgent situations, but may require confirmatory testing with more established methods.

These methods used in blood grouping, specifically for detecting red blood cell agglutination reactions, which are important for determining blood type and compatibility for blood transfusions.

✓ *Slide Test*

In this test, a drop of the individual's blood is placed on a glass slide and mixed with anti-A and anti-B antibodies. The slide is then gently rocked, and the presence or absence of agglutination (clumping) is observed under a microscope. If agglutination occurs with anti-A antibody, the blood type is A; if agglutination occurs with anti-B antibody, the blood type is B; if agglutination occurs with both antibodies, the blood type is AB; and if no agglutination occurs, the blood type is O.

ISSN No:-2456-2165

✓ *Tube Test*

In this test, the individual's blood is mixed separately with anti-A and anti-B antibodies in test tubes. The tubes are then centrifuged to promote agglutination, and the presence or absence of agglutination is observed.

✓ *Microplate Test*

The microplate test is a variation of the tube test, where the individual's blood is mixed with anti-A and anti-B antibodies in wells of a microplate instead of test tubes. The microplate is then centrifuged, and agglutination is observed in the wells. These tests are commonly used in high-throughput settings where multiple samples need to be tested simultaneously.

✓ *Column or Gel Centrifugation Test*

In this method, gel particles coated with anti-A and anti-B antibodies in a column. The individual's blood is added to the top of the column, and the column is then centrifuged. If agglutination occurs, the red blood cells get trapped in the gel column, indicating the blood type.

These tests are performed in a clinical laboratory by trained medical professionals and are important for accurately determining blood type and compatibility for safe blood transfusions and other medical procedures involving blood products.

➢ *Limitations*

The conventional method of blood grouping has been in use for over a century and is widely used in clinical practice. However, there are several challenges associated with this method:

- *Limited Blood Group Typing*

The ABO blood typing system categorizes blood into four main groups - A, B, AB, and O. However, it does not provide information about other blood group systems, such as the Rh system or other minor blood group antigens, which can be clinically relevant in certain situations, such as during pregnancy or blood transfusion in patients with rare blood types.

- *Risk of Transfusion Reactions*

Inaccurate blood typing can lead to transfusion reactions, which can range from mild to severe, including life-threatening hemolytic reactions. ABO-incompatible blood transfusions can result in severe hemolytic transfusion reactions, leading to patient morbidity and mortality.

- *Lack of Automation*

The conventional blood grouping method relies on manual testing, which can be labor-intensive and prone to human error.

- *Risk of Contamination*

Blood typing involves handling of blood samples and reagents, which can carry the risk of contamination. Contamination of samples or reagents can lead to inaccurate blood typing results, potentially resulting in transfusion reactions or other adverse events.

- *Human Error*

Blood grouping is dependent on human involvement, and errors can occur during sample collection, handling, labeling, and interpretation of results. Human errors, such as mislabeling of samples, misinterpretation of agglutination reactions, or transcription errors, can lead to incorrect blood group identification, which may result in transfusion of incompatible blood or other medical errors.

- *Limited Resolution*

Some methods may have limited resolution, meaning they may not be able to accurately identify rare or variant blood groups. For example, there are numerous rare blood group types that may require specialized testing or molecular techniques for accurate identification, which may not be routinely available in all laboratories or clinical settings.

- *Time-consuming*

Traditional methods, such as serological methods or PCR-based techniques, may require several steps and hours to complete, which can be time-consuming, especially in urgent or emergency situations. Delayed results may affect patient management decisions or transfusion timeliness, and may not be ideal in time-critical scenarios.

- *Resource Intensive*

Some methods, such as PCR-based methods or automated analyzers, may require specialized equipment, reagents, and trained personnel, which can be resource-intensive and may not be readily available in all healthcare settings, particularly in resource-limited or remote areas. This may limit the accessibility and availability of accurate blood group identification, especially in underserved populations.

In addition, the patient feels discomfort when blood samples are taken from them in a clinical setting. In rare circumstances, homophobic patients may experience anxiety episodes in addition to symptoms like nausea and fainting. 2.5% of people experience fainting after or during blood withdrawal, according to statistics. Additionally, individuals who have high blood pressure or other blood-related illnesses, such as hemophilia, may experience significant blood loss from needle punctures and bruising at the puncture site. Lacerations, vascular damage, and improperly sanitized needles can all result in a higher risk of germs getting into the body. When using improperly cleaned syringes, the transmission of bloodborne pathogen diseases including HIV, malaria, and syphilis can increase.

Despite these challenges, the conventional blood grouping method is still widely used and considered reliable when performed accurately by trained personnel.

➢ *Advancements*

Blood group detection has now been made non-invasive methods to recent advances in medicine. Since this strategy is novel, there weren't many resources accessible to analyze it. On the basis of the techniques employed, the following will analyze the pertinent literature.

- *Voltage Detection*

These methods involve analyzing the interaction of light with blood or tissue samples to detect blood group markers. Optical methods can be non-invasive, rapid, and may offer the potential for real-time, label-free blood group identification without the need for sample collection or sample processing. It involves using a microfluidic device that measures the electrical impedance of blood samples. The principle behind is that different blood types have different electrical properties due to the variation in the surface charge of RBCs.

When an AC voltage is applied to a blood sample, RBCs in the sample behave as dielectric particles, and their movement in response to the applied electric field results in changes in the electrical impedance of the blood sample. These changes in impedance can be measured and analyzed to determine the blood type.

The microfluidic device used for this method typically consists of microchannels or chambers through which the blood sample flows, and embedded electrodes that apply the AC voltage and measure the electrical impedance. The blood sample is mixed with a buffer solution to create a homogeneous suspension of RBCs, and then it is introduced into the microfluidic device. The applied voltage induces RBCs to move within the microchannels or chambers, and the changes in impedance are measured in real-time. By comparing the impedance profiles obtained from the blood sample with known impedance profiles of different blood types, the blood group of the sample can be determined. Each blood type (A, B, AB, O) has a unique impedance profile due to the differences in the surface charge and size of the RBCs, allowing for accurate blood grouping.

The process is choosing an LED with the appropriate wavelength, allowing it to fall on the finger, and using it as a light source. The optical signals obtained from the finger are obtained using an optical detector (OPT101). The voltage value from the detector varies depending on the optical characteristics of the blood type. Therefore, ABO blood typing is determined by voltage variations [6].

The data bank for 100 people's blood groups was developed with the aid of the prototype. People are urged to insert their middle finger into the arrangement to give them a comfortable position. Each person underwent two trials, and for each trial, the middle fingers on the left and right hands were scrutinized for the acquisition of the variable output voltage range for the ABO Blood group. According to the information gathered, the changing voltage range for the ABO blood group was identified in this way. The results are shown in Table 1. The sole visual characteristic that is taken into account in this for the determination of blood type is absorption. If additional optical characteristics such as scattering and reflection are taken into consideration, we may quantify the additional blood group component and so distinguish between positive and negative blood groups [7].

Table 1 Voltage Levels of each Blood Group

| Blood Group | Voltage Level |
|---|---|
| O | 0.53-0.55 |
| A | 0.56-0.58 |
| B | 0.59-0.60 |
| AB | 0.61-0.62 |

- *Spectroscopy*

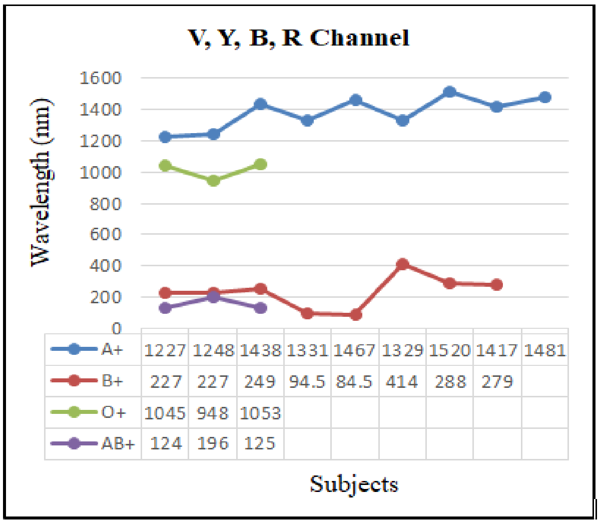
NIR spectroscopy can be used for blood grouping by measuring the characteristic absorption spectra of blood samples in the near-infrared region of the electromagnetic spectrum. When NIR light is passed through a blood sample, it is absorbed by the different chemical components present in the blood, including hemoglobin, water, and other biomolecules. The absorbed light is then reflected back, and the reflected light is analyzed to determine the absorption spectrum.

The absorption spectra of blood samples from different blood types, such as A, B, AB, and O, and Rh+ and Rh- types, show distinct patterns due to the differences in the chemical composition of the antigens on the red blood cell surfaces. These spectral differences can be analyzed using multivariate statistical methods, such as principal component analysis (PCA) or partial least squares (PLS) regression, to develop predictive models that can classify blood samples into different blood types.

In this, a NIR spectrometer is used, which typically consists of a light source that emits NIR light, a detector that measures the intensity of reflected light, and a computer system for data analysis. The blood sample is usually prepared by placing a small droplet of blood on a glass or plastic cuvette, which is then inserted into the NIR spectrometer. The NIR light is passed through the blood sample, and the reflected light is measured and analyzed to determine the blood type based on the established predictive models.

The microcontroller is given 5V of input voltage to initialize. Index finger is positioned and the sensor reads the corresponding wavelengths after the LED turns on. The acquired wavelengths are evaluated and shown on an OLED display.

Data on 25 subjects in all were gathered and summarized. The sensor outputs six channels with wavelengths of 610, 680, 730, 760, 810, and 860, respectively, which are denoted by V, B, G, Y, O, and R. The test entails inserting the index finger in such a way that light passes through it and the fluctuations of blood in the finger are measured by the sensor on a total of 10 people, including both male and female. For the aforementioned analysis, 6 sensor channels with wavelengths of 610, 680, 730, 760, 810, and 860 nm were used. The data acquired from the six channels are discovered to be in declining order, with the wavelength value decreasing as it advances from 610 nm to 860 nm. The values obtained for each participant nearly match up across all 10 fingers. The wavelength values for A+, B+, O+, and AB+ are obtained in the V, Y, B, and R channels at 610, 760, 680, and 860 nm, respectively. The graphical representation of data is shown below in Graph 1.




Graph 1 Wavelength Representation of each Blood Group

- *Biofluids*

✓ *Saliva*

Saliva contains traces of blood group antigens and antibodies, and saliva-based methods have been developed for blood group detection. These methods typically involve collecting saliva samples from the individual and analyzing them for the presence of blood group antigens or antibodies using techniques such as enzyme-linked immunosorbent assays (ELISAs) or molecular methods. Saliva-based methods offer the advantage of non-invasiveness and may be suitable for point-of-care testing or remote settings.

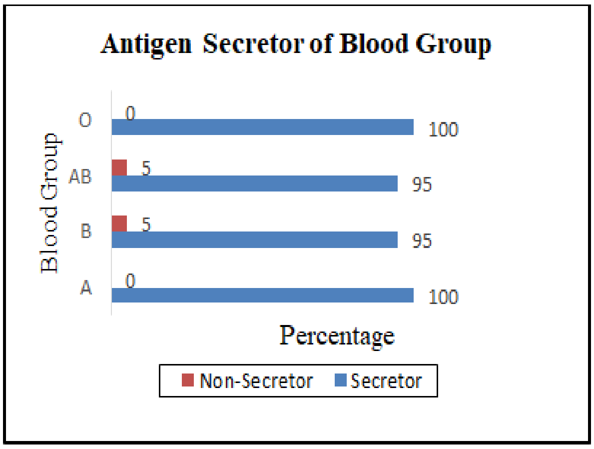
✓ *Urine*

Urine also contains traces of blood group antigens and antibodies, and urine-based methods have been explored for blood group identification. Urine samples can be collected non-invasively and analyzed for the presence of blood group markers using techniques such as ELISAs, lateral flow assays, or molecular methods. Urine-based methods may offer simplicity and ease of sample collection, particularly in situations where blood sample collection may be challenging or undesirable.

✓ *Buccal Swab*

Buccal swabs involve gently scraping the inside of the cheek to collect cells for analysis. Buccal swab-based methods for blood group detection have been developed, which involve collecting buccal swab samples and analyzing them for the presence of blood group antigens or antibodies using techniques such as PCR, microarrays, or other molecular methods. Buccal swab-based methods are non-invasive, easy to perform, and may be suitable for point-of-care testing or remote settings.

It was discovered that 80 percent of people are secretors, which means they release antigens into their bodily fluids [1]. It was determined in a study involving 80 participants that blood categories A and O were absolute secretors 100% of the time and blood groups AB and B were secretors 95% of the time [2]. The results are represented in Graph 2. The antigens may or may not be secreted by particular blood types, and not all people are secretors. Therefore, not all patients can benefit from this therapy. Additionally, the research's validity cannot be guaranteed due to the insufficient sample numbers used. Despite the fact that the approach is completely non-invasive, using chemical testing would lengthen the time it took to identify the blood group.




Graph 2 Secretor Information of each Blood Group

➢ *Artificial Intelligence*

AI and machine learning algorithms are being developed and integrated into blood group identification methods to improve accuracy and efficiency. AI-based approaches can analyze large datasets and complex patterns to aid in blood group identification, interpretation of results, and decision-making. AI can also assist in automating data analysis, result reporting, and quality control, which may enhance the speed and accuracy of blood group identification.

Image processing is a technique for applying various procedures to an image in order to improve it or extract some relevant information from it. It is a kind of signal processing where the input is an image and the output can either be another image or features or characteristics related to that image. Image processing basically includes the following three steps:

- Importing the image
- Analyzing and manipulating the image
- Output based on image analysis

Based on the presence or lack of antigenic components on the surface membrane of the red blood cells, the system uses a light scattering technique to automatically classify blood cells [3]. Antigenic materials and antigen epitopes are traversed by light from the optical device, allowing light to entirely pass through, deflect, diffract, and reflect. A camera is used to identify this scattered light by taking several pictures of a specific focal area. Based on the dispersed light pattern that the camera picked up in these photographs, the blood type is revealed.

ISSN No:-2456-2165

The optical gadget is put on the patient's fingertip and turned on to fire multi-wavelength light onto the skin surface. When light is reflected or absorbed by the red blood cells, the sensitive photo-detector follows the path of the light. Hemoglobin in red blood cells absorbs light when illuminated at specific frequencies, and because illumination is coherent, light is also somewhat dispersed when it hits antigenic determinants with particular structures or shapes. By keeping the optical device on for a predetermined amount of time to capture the effects of scattering, the pattern of this light scattering is captured. The gadget then takes a series of photos to trace the dispersed light. The MWL is highly sensitive to size, shape, and composition of RBC occurring due to the presence of epitopes of various antigens, and image processing algorithms recognize these scattering events and record the pattern/distribution of scattered light, which depends on the molecular shapes of epitopes of various antigens like antigen A, antigen B, and Rh antigen [5]. The obtained dataset is then compared to the observed pattern to estimate the blood type.

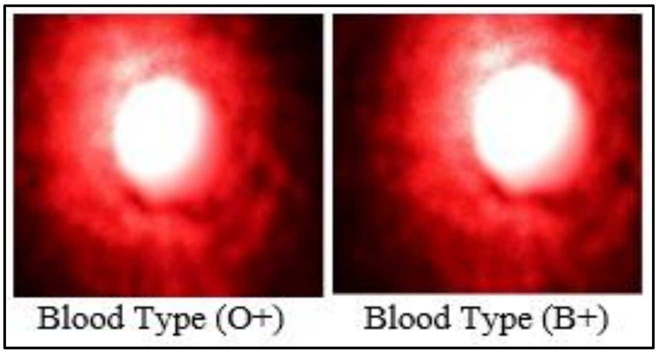



Fig 1 Images of Blood Group Taken Via Image Processing

## II. DISCUSSION

It can be concluded that the visible light-voltage detection method of blood grouping had the highest reliability out of all literature that was studied because it required less time for detection. Due to the constraints put forth, no prototype has yet been shown to society, and all models are still in the preliminary trial stages. To accurately measure light that is detected after travelling through several layers of the body, the instrument's sensitivity must be very high. As a result, it will become more expensive to produce and less appealing. The results will also vary depending on the morphology. The sample sizes applied for deep learning and image processing methods were not specified. Despite its benefits, the deep learning method's validity could not be determined because the outcomes were not made public. Based on the results, it was determined that the NIR photon transmission method that was discussed had the highest accuracy and detection speed. The Rh factors were also taken into account, but the validity of this method's accuracy was not sufficiently tested because there was a dearth of relevant literature and a small sample size. The effectiveness of the bodily fluid and antibody-based detection techniques was not well supported by the evidence, and they took a long time to get results for all blood groups.

Both NIR spectroscopy and the voltage detection method have been demonstrated to be useful for blood grouping, however the accuracy of each method may differ based on the particular application and tools employed. Studies have shown that NIR spectroscopy can reliably identify blood groups with accuracies ranging from 80% to over 90%. However, it may be more prone to interference from elements such sample variability, antibody quality, and electrode location with the voltage detection approach.

It's important to note that non-invasive technologies are still being researched and developed, and may not be widely available or validated for routine clinical use in all settings. Further studies and validations are needed to establish their accuracy, reliability, and clinical utility. However, these new and upcoming technologies hold promise for potentially overcoming some of the limitations of traditional blood group identification methods and improving the accuracy, efficiency, and accessibility of blood group detection in the future.

## III. CONCLUSION

Blood group identification is a critical aspect of clinical practice, as it plays a crucial role in blood transfusion, organ transplantation, and forensic investigations. Traditional methods of blood group identification require invasive procedures involving blood sampling, which can be uncomfortable, time-consuming, and pose risks such as infection and allergic reactions. Non-invasive methods of blood grouping, which do not require drawing blood from a person's vein, can offer several advantages in various clinical and practical settings. These methods may require fewer resources and equipment compared to invasive methods. For example, fingerprint-based blood grouping methods can be rapid and straightforward, as fingerprints can be easily acquired using fingerprint scanners or other non-invasive devices, and the analysis can be automated using machine learning algorithms. These methods have the potential for point-of-care testing, where blood typing can be performed at the bedside or in remote locations without the need for specialized laboratory facilities. This can be particularly advantageous in emergency situations or in settings where immediate blood typing results are critical for patient care, such as in rural or remote areas or during disaster response scenarios.

In recent years, there has been growing interest in developing non-invasive methods for blood group identification that are safe, convenient, and efficient. In this literature review, we summarize the current state of research on non-invasive methods for blood group identification, highlighting their advantages, limitations, and potential applications.

It is important to note that non-invasive blood grouping methods are still in the research and development stage and may have limitations in terms of accuracy, reliability, and widespread availability. Further validation and testing are needed to establish their clinical utility and safety. However,

ISSN No:-2456-2165

the potential advantages of non-invasive blood grouping methods make them an area of active research and innovation in the field of blood transfusion medicine and clinical diagnostics.

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