SciDoc Publishers
Infer, Interpret & Inspire Science

International Journal of Forensic Science & Pathology (IJFP)
ISSN: 2332-287X

ABO Typing In Forensic Analysis: to be or not to be in the epoch of genotyping

Review Article

Sandip Ghosh*

Assistant Director, Biology Division, Forensic Science Laboratory, Government of West Bengal, West Bengal, India.

Abstract

Increasing crime rate, in the modern era, has prompted the scientists to develop fast and effective forensic strategies to distinguish between guilty and innocent. Since ages, investigators have relied on ABO typing which was the only method available mostly to exclude the innocent from the suspect list, though most of the ABO-typing methods are low throughput and time consuming. Moreover, as the aim of every investigation is to establish the identity of the offender, the ABO typing methods, being used, even if done properly, were proven to be non-beneficial to single out the perpetrator and thus futile in the court of law. Due to lack of accuracy, specificity, conclusiveness and availability of sample, ABO typing is fast being replaced by genotyping, which is a more suitable method to convict the offender. The pros and cons of different ABO-typing methods and the usefulness of genotyping techniques in this context have been discussed here. In spite of being overshadowed by genotyping, ABO typing is still being used as a tool in forensic science in developing countries because of its cost-effectiveness. However, it is concluded that the potential challenge for forensic investigators to identify suspects possibly poses a future, where genotyping will be the ultimate tool and ABO typing may become obsolete.

Keywords: ABO-Typing; Absorption-Inhibition; Absorption-Elution; STR-PCR; Touch DNA Analysis; Epigenomics.

Introduction

Forensic science is an interdisciplinary field that uses scientific knowledge from varied fields like biotechnology, toxicology, chemistry, physics and others to analyze and characterize physical evidence found at the scene of crime. In criminal matters, particularly those involving violence, specimens of blood, semen, and other body fluids or tissues are used as evidence as they provide information that may solve the case. Blood is considered one of the most important biological samples that are frequently obtained from the crime scene. It is regarded as a very important forensic tool since analysis of different aspects of bloodstains can provide valuable information which helps the investigators to have a clear understanding of the circumstances under which a crime has been committed. The use of blood in forensic analysis is a method for identifying individuals suspected of committing a crime, solving disputes in paternity etc [1].

The term Forensic serology has generally been used to refer to the identification and individualization of biological evidence, including all the activities and tests associated with the evaluation and typing of biological evidence in criminal matters. The word serology was derived from serum, the fraction of blood containing antibodies. Blood grouping was long the only means of individualizing biological evidence, and serology classically encompassed blood groups and blood typing [2].

Origin and ABO typing

The importance of blood grouping in medico-legal issues, lies in the fact that blood groups are considered genetic markers since they are strongly inherited following the Mendelian laws, and are unaffected by environmental factors such as nutrition,diet, age, occupation, diseases. It can withstand stringent conditions, such as high heating or drying [3, 4] and thus remain unchanged throughout life [5] Blood grouping is the classification of erythrocytes or red blood cells (RBCs) based on surface markers or antigens like A, B, D, H, etc., present on their cell membrane [6]. There are more than 300 antigens present on the RBC membrane and based on these antigens, 38 blood group systems have been identified by

***Corresponding Author**:
Sandip Ghosh,
Assistant Director, Biology Division, Forensic Science Laboratory, Government of West Bengal, West Bengal, India.
Tel: 91-033-25565430/91- 9434706988
Fax: 91- 033-25565430
E-mail: sandip@rocketmail.com

Received: February 25, 2022
Accepted: March 28, 2022
Published: March 30, 2022

Citation: Sandip Ghosh. ABO Typing In Forensic Analysis: to be or not to be in the epoch of genotyping. *Int J Forensic Sci Pathol.* 2022;9(3):487-494.
doi: http://dx.doi.org/10.19070/2332-287X-22000101

Copyright: Sandip Ghosh©2022. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

International Society of Blood Transfusion which includes ABO, Rh, P, Kell, MNS, Lewis, Kidd, Diego, etc [7]. Due to complexity, and expense of testing for possible reactions among all known antigens, the simpler ABO and Rh blood typing system remains the primary, cost-effective and conspicuous method in practice for clinical and forensic use [8].

The ABO blood group system, first described in 1901 by Karl Landsteiner is the most basic system which divides blood into four groups, or types: A, B, AB and O [9] Blood group specificity depends upon the inheritance of the ABO and H genes and the subsequent expression of these antigens on the red blood cells. For e.g., an individual having blood group A will express antigen "A" on the surface of the RBCs and will contain "anti-B" antibody in the plasma.The Rhesus-system is the second most imperative blood group system after ABO[10]. This system is based on the expression of the "D" antigen or Rh factor on the RBC membrane and accordingly, the status of a person is indicated as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent). Landsteiner's description of blood types gave a new opening to forensic science and based on this, forensic scientists could definitively compare blood evidence left at a crime scene to the blood of a suspect. The Rh factor enabled forensic scientists to better study the blood of suspects and to potentially exclude individuals as the source of blood at crime scenes and narrow down the list of suspects [11]. Therefore blood-typing could be used to help prove innocence, but not to identify a suspect beyond a reasonable doubt, the standard necessary for a criminal conviction in many criminal courts.

However, accuracy of ABO blood typing is a major concern for the forensic serologist since inception. Small sample quantity, degraded and contaminated blood, hemolysis, putrefaction, mummification and skeletonization of the body during the post mortem [12] often makes the typing method mostly inconclusive either due to confusing observations or due to lack of sufficient stained sample present.

Species identification

Species identification along with ABO- typing of different human samples in forensic serology has long been one of the well accepted test in criminal investigation [13]. Beside blood, several other evidences, hair, semen and saliva may present in the crime scene, which can be used to link the suspect in accordance with the Locard's principal.

The amount of biological evidence collected at a crime scene is often extremely small, and thus it becomes particularly important for the forensic scientists to preserve as much of the evidence as possible and select the accurate tests for analysis that require trace amounts of sample.

After detection, the sample requires the test for species identification. Species identification is important prior to ABO-typing because the forensic materials may be contaminated with the animal body fluids. For most of the body fluids the primary analysis for species determination is by 'precipitin' test [14] which used the principle of interaction between antigens and the anti-sera raised against the blood cells of the same species. Precipitin tests can be performed in various ways. In every instance, however, the goal is to bring the antigens and antibodies, both in solution, into contact with one another.

The earliest precipitin test method was the "ring test," where an aqueous solution of antigen is layered over the more dense antiserum solution in a tube. The formation of precipitate at the interface between the layers indicates a positive test. This method was largely abandoned when gel-based methods, such as Double Diffusion' [15] was developed, which was also based on the same principle. Though used globally these methods have some limitations. First, due to unavailability of enough quantity of sample it is always difficult to titrate the required quantity of antigen-antibody to get a visible result and the second, is to eliminate the cross-reactivity of antisera [16] against one species with bloods of evolutionary closed species (e. g., human &chimpanzee). Both the limitations are some-way related to the particular batch of antisera being used. Therefore, it is always necessary to test the new batch of antisera every time against the homologous blood. The precipitin test can also be done using the antisera, raised against the hemoglobin of the species, which detects the origin of species as well as confirms the presence of blood. Unfortunately, the blood stain often found in the crime scene is dried and is not a true mixture of blood cell and serum. Depending on the environment and clotting time (before drying or after drying) the ratio of cell/serum often varies.The antisera raised against serum or hemoglobin will therefore, produce different results for different cell/serum mixture for the same species.

Nevertheless, the "anti-human" sera raised against human serum proteins react with some human physiological fluids, e. g. human semen, saliva, and semen-free vaginal swab extracts although to a lesser extent than they do with serum or blood. Therefore, the anti human sera can be used to test for human proteins in biological fluids other than serum. It must also sometimes be considered in the interpretation of positive test results when the specimens are or may be mixtures of blood and/or various physiological fluids. There are a number of more involved technical modifications of precipitin tests that have been proposed or used, particularly to help in differentiating closely related species [17].

ABO - Typing

Agglutination is the main criteria to detect antigen-antibody interaction, using the principle of immunochemistry.

The main avenues for ABO typing are (1) Direct method and (2) Indirect technique. The direct method could be classified into two types ; (i) Forward method, depending on the presence/absence of antigen [18] on the cell surface, & (ii) Reverse method depending presence/absence of antibody in the serum part [19] The indirect method was mainly the antigen-antibody combined technique, for which two basic methods have been reported namely, absorption-inhibition [20, 21] and absorption-elution [22, 23]. Both these methods are used to detect the blood group antigens remaining on the ruptured cell membrane. Some other high throughput methods, such as Enzyme linked immuno-sorbent (ELISA) assay [24, 25] and Micro-plate method [26] have also been tried. The latter two methods, when used in combination with absorption-elution, helped to increase the efficiency of the result.

Forward method

Table 1. Limitation of different ABO-typing methods.

ABO –Typing Method	Limitation
Direct Agglutination	1. Unsuitable for old, dried stain 2. Antigenecity may be lost by bacterial contamination
Lattes Method	1. Unsuitable for old stain 2. Antibodies present in serum are relatively unstable
Absorption-inhibition	1. Time consuming 2. Low throughput 3. Extensive sample handling 4. Often inconclusive for aged, dried stains 5. Need to know optimum antisera dilution prior to the method 6. Indirect observation
Absorption-elution	1. Time consuming 2. Low throughput& difficult to automate 3. Specialized skill needed 4. Often not conclusive for dried, aged stains 5. Indirect observation

Table 2. Advantage of genotyping over ABO-typing.

ABO -Typing	DNA typing
1. Relatively more quantity of sample needed.	1. Minute quantity is enough.
2. Unable to determine sex	2. Able to determine sex
3. It can be used as a presumptive test to include or exclude the suspect	3. It Is a confirmatory test to convict the offender.
4. Most of the typing methods are low throughput, which requires specialized skill to get conclusive result.	4. High throughput and robotics can be used to get conclusive result.
5. Mostly fails in cases where touch –exhibits, e.g skin cells are the only source to link the suspect to the crime scene	5. Useful for Touch-DNA analysis from the crime scene or object to link the suspect to the crime scene.
6. Not useful for paternity determination.	6. Paternity determination is possible.
7. Difficult for making any conclusion for multiple accused sample	7. Individualization of the offender from the multiple accused is possible.

It is the simplest ABO-typing method, which is mainly used for the fresh blood. The antigen on the cell surface and anti-sera raised against the blood antigen reacts together and a cell-cell attachment occurs through antigen-antibody bridge. This cell-cell attachment (agglutination) can be visualized in the microscope. The efficiency of agglutination depends on the proper cell structure, antibody binding capacity, osmotic fragility etc [27, 28]. In dried, aged stains the antigens are partially or completely destroyed. Sunlight and humidity are also known to be destructive for the antibody stability [29]. Moreover, hemolysis or partial hemolysis has been a common observation during suspension of cells in saline, although, later it was shown that the hemolysis could be partially avoided by using 40% saccharose solution of pH 6 [30].

Lattes method

An alternative reverse approach has been reported by Lattes [19] based on serum antibody. It used the unique property of the ABO system, that an individual's serum contained antibodies corresponding to the antigen that he or she lacked. This process has its own limitations for the analysing forensic samples as the serum antibodies are far less stable in dried stains. Though some laboratories reported the presence of antigen in the dried stain, they could not opine the antigen type conclusively which may be due to less sensitivity of the method. Mistyping can also occur in the stain having some other unrelated antibodies in the serum, which causes the agglutination of the test cells. The possibility of misinterpretation of result has been a common observation in report using older cells.

Absorption-inhibition

Absorption-inhibition was the first combined method reported by Schutze, 1921, where he used the dried blood sample for ABO typing. The method was less sensitive, which needed the predetermined titer value of the anti-sera to work with. A two-dimensional absorption - inhibition method [21] was proposed, which took best features of inhibition-titration and titration-inhibition at the same time and was shown to be more sensitive than either of them.

Absorption-elution

The absorption-inhibition method was subsequently replaced by Absorption - elution method [22] and was consequently refined by the method developed by Kind (1960), which was predominately used by most of the laboratories for its high sensitivity. Though ABH antigen detection was reported even for 42 weeks aged samples by absorption –elution method [4], some discrepancies were also observed [12]. In this context, a comparative study

between direct agglutination and absorption-elution methods was performed, which showed some cross reactivity in absorption-elution method for the blood stored at room temperature for 60 days [28]. That result could not comprehensively eliminate the possibility of mistyping for the aged, dried stains [31] even using absorption-elution method. Haemolysis might also produce negative results [32]. Though, ABH antigens were shown to be stable [3] at high heat, activity changes was reported for the dried blood stains on standing [4]. Surface type (cotton, synthetic fabrics, wooden surface) was also shown to affect the efficiency of this method with age [33]. High sensitivity of this method can also be a disadvantage as some other body secretions like, semen, saliva which are also the source of ABH antigen might interfere with the result.

However, all ABO typing methods for dried, aged blood stains, were complex, tedious, time-consuming and low throughput, which were difficult to automate. As it involved, extensive sample handling, and complex techniques, which were coupled with human skill and experience, a lot of discrepancies and false positive/negative results were also found [12]. The method also became vulnerable while determining the blood group of wet stains and/or decayed bodies [12] and possibilities of mistyping occurred in aged blood sample [4]. The failure might be attributed to the loss of antigenicity or to the acquired antigenicity by bacterial contamination [34] and/or partial digestion of erythrocyte membrane by proteolytic enzymes [35]. All these limitation made most of the methods unreliable to work with.

ABO typing in other body fluids

In the 1930s, it was found that the blood group antigens, were not confined to erythrocytes but were also present in various other body secretions and tissue fluids, such as saliva, semen, sweat, urine, vaginal secretions, etc., from which blood groups could be determined [36], provided the subject had the secretor status [37, 38]. The status of an individual as secretor or non-secretor is determined by the presence of Lewis antigens, Lea and Leb, which are not intrinsic to the RBC membrane but are synthesized by intestinal epithelial cells and circulate in plasma [39, 40]. The ABO group specific substances are typically present in high concentrations in body fluids of secretors and the secreted substances can withstand drying and retain their antigenic activity over a prolonged period. The forensic serologists took advantage of these characteristics to group stains of body fluids such as semen [41] and saliva [42] that assisted in the investigation of crimes where there was lack of blood sample. Studies on the secretor status of blood group antigens had extensively been studied for saliva [37, 38, 43] vaginal sample [44], sweat stains [45] and semen [46]. ABH antigens were also expressed in some indoor pets also [46], so the secretion from the saliva of rabbit, cat, dog interacted with the antibodies raised against ABH antigen [47]. It is therefore important to exclude false positive reaction and mis-judgement of species identification in the blood group determination.

Due to unavailability of adequate techniques, analysis of anti-A and anti-B haem-agglutinins in saliva or other body fluids were not utilized as evidence in the medico legal cases initially [48]. Later, a lot of modifications were done to develop highly precise techniques to determine blood group antigens in body fluids. At present, two methods are used to type blood and body fluids for ABO grouping, the absorption-inhibition (AI) and the absorption-elution (AE) methods. Absorption-inhibition showed a 100% positive correlation for ABO blood grouping in dried salivary samples and was found to be a more effective technique for recognizable proof and secretor status determination in different populations [49]. A different study carried out the estimation of blood group antigens on cigarette butt [50] also showed the Absorption-inhibition method to be more fruitful. But in a comparative study [42] absorption-elution technique was demonstrated to be more sensitive, specific, and effective in determination of ABO blood grouping in dried saliva stain.

ABO-typing from other tissue

ABH antigen is expressed in human cells including dermal epithelium, endothelial cells of blood vessel Hassall's corpuscle [51], renal tubules, secretary cell of respiratory tract [52], and male reproductive organs [53]. Indirect typing method were mostly tried for hair [54, 55] tooth [56-59] and bone tissue [60, 61]. Some histochemical techniques have also been used for hair [52, 62] and other tissue [53]. However, the histochemical method is very time consuming which requires a lot of skill dependent steps to reach to a conclusive result.

Advent of DNA technology

Unfortunately, the limitations of ABO-typing methods (Table 1) for forensic samples along with variable reports by different laboratory, made the results inconclusive in many cases. The effect of autolysis, dehydration, loss of antigens by temperature, humidity, and aging, or contamination by microbes or animals may haveled to variations in the study. Moreover, even though, properly done, it did not always have the potential to produce concrete evidence to individualize the offender and therefore may be proven futile in the court of law.

DNA Fingerprinting

Forensic science is nothing but the art of piecing together a crime scene in order to determine how the crime was committed and who was responsible. DNA evidence is one of the most prominent pieces of evidence, which is lacking in ABO typing, method to individualize the perpetrator.

Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphisms, or RFLPs as they are commonly known, were the first type of DNA fingerprinting which came onto the scene in the mid-1980's [63]. that focus on the size differences of certain genetic locations. Some other methods, utilizing the RFLP principle were used, such as, amplified fragment length polymorphism [64], and terminal restriction fragment length polymorphism [65].

Variable Number Tandem repeats VNTR

Variable number tandem repeats, or VNTRs represent specific locations on a chromosome in which tandem repeats of 9-80 or more bases repeat a different number of times between individuals. These regions of DNA are readily analyzed using the RFLP approach and a probe specific to a VNTR locus. The fragments are a little shorter than RFLPs (about 1-2 kilo base pairs), but are created through the exact same process.

STR-PCR analysis

After the invention of Polymerase Chain Reaction by Mullis [66], the DNA fingerprinting is largely being done by STR marker analysis [67-69]. Unlike VNTRs which analyze mini-satellites that have repeat of 9-80 base pairs STRs use microsatellites which have repeat sequences of only 2-5 base pairs [70], tandemly repeated at a specific locus, mostly in the non-coding regions , introducing the "less is more" philosophy to the world of DNA fingerprinting. This was a big step forward in forensic science since the length of DNA fragment being analyzed is short enough to be amplified by polymerase chain reaction (PCR), which enabled the analysis of a very small sample of DNA. This method is quicker and easier than any previously known method and match it to a person's identity. The STR typing method is nowadays mostly performed by the robotics starting from, DNA extraction, amplification (PCR) and all through DNA profiling. That is why, a skilled or trained personnel may not be needed to perform the analysis, though the interpretation part would still require some knowledgeable person in this field.

Next Generation Sequencing (NGS)

Next generation sequencing (NGS) was another landmark in DNA fingerprint analysis, which is capable of sequencing thousands of genomic regions of a species simultaneously to get idea about the phenotype, age of the unknown source retrieved from the crime scene [71]. A promising NGS approach was reported with simultaneous analysis of 10 STRs, 386 ancestory-specific SNP s, and the complete mtDNA [72]. Other two studies [73, 74] has been reported, where they analysed 27 autosomal, 7-X chromosomal, 24-Y chromosomal, 94 identity specific SNPs and 56 ancestory informative SNPs in a single sequencing. The method has been shown to be applicable in degraded and low template samples [75] as well.

Rapid-Hit technology

This is also a high-throughput method which does not require any human intervention in whole process. It takes help of the simple "sample in-profile out' principle and analysis is done in a single step, including extraction, amplification and sequencing within 2 hrs [76]. The minimum time taken for genotyping makes it very useful for the criminal justice system and has a tremendous potential to use it at the crime-spot, to include/exclude the suspect. This method is currently under validation for law enforcement use.

Merits & demerits of DNA- typing

The crime rate around the globe is increasing alarmingly and the need for fast and effective methods of crime and criminal detection is also increasing. As the criminal justice system comes of age, it really requires individualization of a suspect from a crime scene. ABO-typing could be considered as the evidence which was able to place an individual in a general class but failed to identify the offender conclusively. For example, blood typing can be used to establish whether someone has type A, B, AB, or O blood, which can be useful in helping to investigate a crime by either including or excluding an individual from the list of suspects but cannot pin down the actual culprit. On the contrary, individual evidence, such as fingerprints and DNA, can be used to unravel the identity of an individual. DNA analysis has made possible the accurate typing of very small traces of body fluid evidence to a very high level of individuality in many cases. A laboratory worker might be virtually certain, therefore, that a biological trace has originated from a particular person.

Apart from the advantages over mere ABO typing (Table. 2) the DNA typing has a vast potential in the criminal justice system in future. Touch DNA is one of them. It was a break-through technology developed [77-79], which did not require any biological fluids to type DNA, instead it could isolate the DNA from the touch site of the object having the skin epithelial cells of the accused remain at the crime scene. When a crime is committed, the offender often may touch items, like weapon, clothing, victims body, etc [80], which were used as a DNA source of the perpetrator. It helped massively in investigating "cold cases" which were closed due to lack of evidence.

However, STR profile data was found to be ineffective, where the suspect is unknown and any genetic data was unavailable to compare with. In those cases, DNA profile was compared with those in data banks, kept in many state and nationally for the offender, which could link the perpetrator to the crime scene. For non-matching DNA profiles, a statistical approach [81] was proposed. The ancestry-informative SNP marker PCR multiplexing was also an effective method to infer the geographic background of the donor by using five major sub-continental population groups [82]. New investigative lead was also provided using uni-parentally inherited markers (mitochondrial DNA and non-recombinant Y-chromosome) to infer the ancestries of the maternal/paternal background of a DNA sample and thus, helped to narrow down the suspect list.

Another, limitation what the forensic scientists often encounter is the inability to determine the age from the skeletal remain, since a lot depends on the age of person in question. Though the morphological analysis of the skeleton was the only way [83] using mostly hard tissues, such as,tooth, bone etc, the method have been shown to produce ambiguous result [84]. A promising method for more accurate age prediction arose from the field of epigenomics, where epigenetics and the DNA-methylation markers have been proposed to estimate age, tissue type and even differentiate between monozygotic twins [85]. A significant change of age-related DNA methylation level at CpG dinucleotide islands was shown to be associated with increasing age in epigenome-wide association study [86-88]. Gene expression had also been reported to correlate with human age [89]. But both these method required relatively higher amount of DNA to work with, which is normally absent in the typical forensic specimen [90].

Though omnipotent, DNA typing has some inherent limitation in forensic cases. As it is unable to establish the nature of the biological material which may or may not be important in a particular case. The importance of identification versus typing tests with an evidence item has to be considered in the context of the case. A successful DNA typing on a licked envelope flap or on a swabbing from a bite mark, was likely to be informative regardless of whether saliva could be identified or not. On the other hand, the identification and species-determination aspects of a forensic examination can sometimes be more important for a suspected hit-and-run case. The suspect may be absolved of suspicion by a

finding that bloodstains on his vehicle were of nonhuman origin. In addition, a virtual match between evidence and a person might have little meaning if there is an innocent explanation for the finding. This would be true, for example, when a victim's genetic profile is matched to bloodstains on a suspect's clothing in a case in which the victim and suspect lived in the same household or the suspect is able to offer a plausible explanation for the stains.

Not withstanding the DNA typing revolution, some activities long associated with "forensic serology" remain important and continue to be a vital part of forensic biological evidence analysis. Blood and physiological fluid stains and traces still require identification. More importantly, those aspects of "forensic serology" most characteristic of its association with criminal sciences remain critical if biological evidence analysis is going to help to unravel a case. Recognizing the crucial physical evidence in a given case, using experience and judgment to select the most important and informative specimens for typing in terms of the case, and interpreting stain patterns are essential skills that cannot be replaced by any DNA typing technique.

Conclusions

Degraded samples, loss of antigenecity due to effect of environmental factors, lack of ability to individualize the perpetrator, made the ABO-typing methods unfruitful to the judiciary and rapidly being replaced by high-throughput genotyping. Sex determination and age estimation is often required for the human or skeletal remains found in the crime scene to get a preliminary idea about the crime. Limitation to discriminate between male and female is another feature that makes the traditional. ABO-typing methods inapplicable in various cases. On the contrary, while the STR-PCR can determine the sex, new approaches like, epigenomics can infer about the approximate age of the skeletal remains. Paternity dispute is another area often encountered in both criminal and civil cases, such as, guardianship, inheritance, adultery and fornication, where, ABO–typing is of no use as a conclusive evidence as the A, B, AB and O blood groups are never unique. Genotyping is the only option toconfirm paternity of the disputed off-spring in such cases.Non-secretory status of human subject can also make the other body fluids, other than blood, unsuitable for ABO typing. The problem can be addressed by genotyping where saliva, semen, or other body fluids may be used to individualize a person. In addition, in absence of any detectable biological fluid also, touch-DNA can be handy to get results in presence of skin cell remains in the crime scene. Thus, genotyping, in its present form, mostly fulfils the requirements for the forensic experts, to unravel a case conclusively to court of law. However, in spite of being eclipsed by genotyping, ABO typing is still being used and may be continued to be in use as a routine-tool for forensic analysis in developing countries because of its cost-effectiveness.It is only a matter of time when forensic experts will rely on genotyping methods and the ABO-typing will either be discarded or confined only to a primary screening.

References

[1]. Okroi M, Voswinckel P. "Obviously impossible"—the application of the inheritance of blood groups as a forensic method. The beginning of paternity tests in Germany, Europe and the USA. InInternational Congress Series. 2003 Jan 1;1239:711-714. Elsevier.

[2]. Gaensslen RE. Forensic analysis of biological evidence. Forensic Sciences. 2000;1.

[3]. Nishi K, Tanaka N, Okazaki S, Maeda H, Tsuji T, Nagano T. Effect of heat on blood group A-and B-active glycolipid extracted from AB human erythrocytes. Jpn J Legal Med. 1979;33:86-90.

[4]. Maeda H, Nishi K, Okazaki S, Tsuji T, Tanaka N, Nagano T. Activity changes of blood group antigens in dried blood stains on standing. J Wakayama Med Soc. 1979;30:211-8.

[5]. Shetty M, Premlata K. ABO blood grouping from tooth material. J Indian Acad of Forensic Med. 1972;32:336-338.

[6]. Pourazar A. Red cell antigens: Structure and function. Asian J Transfus Sci. 2007 Jan;1(1):24-32. PubMed PMID: 21938229.

[7]. ISBT (2019). "Table of Blood Group Systems v 6.0 (August 2019)" (PDF). International Society of Blood Transfusion. Archived (PDF) from the original on 27 September 2019. Retrieved 19 January 2020.

[8]. Harbison C. ABO Blood Type Identification and Forensic Science (1900-1960). Embryo Project Encyclopedia. 2016 Jun 2.

[9]. Owen R. Karl Landsteiner and the first human marker locus. Genetics. 2000 Jul;155(3):995-8. PubMed PMID: 10880463.

[10]. Westhoff CM. The Rh blood group system in review: a new face for the next decade. Transfusion. 2004 Nov;44(11):1663-73. PubMed PMID: 15504174.

[11]. Muehlberger CW, Inbau FE. Scientific and Legal Application of Blood Grouping Tests. Am. Inst. Crim. L. & Criminology. 1936;27:578.

[12]. Nishi K, Rand S, Nakagawa T, Yamamoto A, Yamasaki S, Yamamoto Y, et al. ABO Blood Typing from Forensic Materials-Merits and demerits of detection methods utilized in our laboratories, and biological significance of the antigens. Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology. 2005;6(2).

[13]. Ladd C, Bourke MT, Scherczinger CA, Pagliaro EM, Gaensslen RE, Lee HC. A PCR-based strategy for ABO genotype determination. J Forensic Sci. 1996 Jan;41(1):134-7. PubMed PMID: 8934712.

[14]. Uhlenhuth PT. EineMethodezurUnterscheidung der verschiedenenBlutarten, imbesonderenzum differential diagnostischenNachweise des Menschenblutes. 1901.

[15]. Ouchterlony O. Antigen-Antibody Reactions in Gels. 25B Ark Kemi Mineral Geol. 1948;14.

[16]. Nuttall GH. On the Formation of Specific Anti-Bodies in the Blood following upon Treatment with the Sera of different Animals, together with their Use in Legal Medicine. J Hyg (Lond). 1901 Jul;1(3):367-87. PubMed PMID: 20474125.

[17]. Sensabaugh GF. Molecular Evolution and the Immunological Determination of Species. Int. Microform J. Leg. Med. 1976;11:art.219.

[18]. Landsteiner K. Ueber Agglutinationserscheinungen normal enmenschlichenBlutes. 1901 [Agglutination phenomena of normal human blood]. Wien KlinWochenschr. 2001 Oct 30;113(20-21):768-9. German. PubMed PMID: 11732110.

[19]. Lattes L, Bertie LWH. L'Individualità Del Sangue Nella Biologia, Nella Clinica E Nella MedicinaLegale. Individuality of the Blood in Biology and in Clinical and Forensic Medicine... Translated by LW Howard Bertie... from the French Edition of 1929, Thoroughly Revised and Brought Up to Date by the Author. With a Bibliography. Oxford University Press; 1932.

[20]. Schütze H. Hæmagglutination and its Medico-Legal Bearing, with Observations upon the Theory of Isoagglutinins. British journal of experimental pathology. 1921 Feb;2(1):26.

[21]. Lee HC, Gaensslen RE, Pagliaro EM, Novitch B. Two-dimensional absorption-inhibition. J Forensic Sci. 1988 Sep;33(5):1127-38. PubMed PMID: 3193074.

[22]. Siracusa V. La sostanzaisoagglutinabiledelsangue e la suadimostrazione per la diagnosiindividualedellemacchie. Arch AntropolCriminPsichiat Med Leg. 1923;43:362-5.

[23]. Kind S. Absorption-elution grouping of dried blood smears. Nature. 1960 Feb 6;185:397-8. PubMed PMID: 14409131.

[24]. Parigian MJ. An ELISA procedure for the detection of soluble ABH blood group substance in semen, saliva, and vaginal samples. J Forensic Sci. 1995 Jan;40(1):122-5. PubMed PMID: 7876793.

[25]. Lapenkov MI, Gurtovaia SV, AleksandrovaVIu, Kapinos TA. Combination of absorption-elution and gel-filtration methods for group identification of blood spot specimens. Sud Med Ekspert. 2009 May-Jun;52(3):13-5. Russian. PubMed PMID: 19569533.

[26]. Mudd JL. A microplate method for reverse ABO typing of bloodstains. J Forensic Sci. 1986 Apr;31(2):418-25. PubMed PMID: 3711823.

[27]. Tsuji T, Kimura A, Nishi K, Ito N, Mizumoto J, Wada K. Effects of red cell shape changes on hemagglutination. Nihon HoigakuZasshi. 1985 Apr;39(2):138-42. PubMed PMID: 4087512.

[28]. Nishi K, Ito N, Mizumoto J, Wada K, Tsuji T, Kimura A. Reliability of blood grouping of aged blood by two direct hemagglutination methods and the absorption elution method. Nihon HoigakuZasshi. 1985 Apr;39(2):131-7. PubMed PMID: 4087511.

[29]. Wang J, Yiu B, Obermeyer J, Filipe CD, Brennan JD, Pelton R. Effects of temperature and relative humidity on the stability of paper-immobilized antibodies. Biomacromolecules. 2012 Feb 13;13(2):559-64. PubMed PMID: 22257068.
[30]. Michailow R. A method for treatment of partially haemolysed blood with lost agglutination properties, allowing its blood group determination (author's transl). Z Rechtsmed. 1977 Jul 5;80(1):35-8. German. PubMed PMID: 883425.
[31]. Açıkgöz H, Kendi İ, Bilge Y. Environmental Factors Affecting Blood Stains. Ankara University Faculty of Law Journal. 2007; 56(3):1-10.
[32]. Özer A. Practical Hematology Ege University Faculty of Medicine Publications. Izmir: Ege University Printing House Serology. 1980;65-105
[33]. Mishra SK, Yadav AS. Variations in ABH Antigenic Stability of Dried Bloodstains from Different Surfaces with Age. Indian Journal of Forensic Medicine & Toxicology. 2012 Jul 1;6(2):202.
[34]. Shanmugam P, Roy R, Sethi J. Impact of age and microbial growth on the accuracy of preliminary tests and blood grouping of dried blood stains in forensic investigations. Indian Congress of Forensic Medicine & Toxicology.2015.
[35]. Bosmann HB. Red cell hydrolases. 3. Neuraminidase activity in isolated human erythrocyte plasma membranes. Vox Sang. 1974;26(6):497-512. PubMed PMID: 4137161.
[36]. Schiff F, Sasaki H. Der Ausscheidungstypus, ein auf serologischemWegenachweisbaresmendelndes Merkmal. KlinischeWochenschrift. 1932 Aug;11(34):1426-9.
[37]. Motghare P, Kale L, Bedia AS, Charde S. Efficacy and accuracy of ABO blood group determination from saliva. Journal of Indian Academy of Oral Medicine and Radiology. 2011 Jul 1;23(3):163.
[38]. Saboor M, Ullah A, Qamar K, Mir A, Moinuddin. Frequency of ABH secretors and non secretors: A cross sectional study in Karachi. Pak J Med Sci. 2014 Jan;30(1):189-93. PubMed PMID: 24639859.
[39]. Grubb R. Correlation between Lewis blood group and secretor character in man. Nature. 1948 Dec 11;162(4128):933. PubMed PMID: 18104581.
[40]. Henry S, Oriol R, Samuelsson B. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang. 1995;69(3):166-82. PubMed PMID: 8578728.
[41]. Verma RP, Arya E. Determination of serological markers (blood group markers) of biological fluid (semen) obtained from crime scene for individualization of the donor (s). Int J SciEng Res. 2014;5:2307-1.
[42]. Sen MP, Vanishree M, Hunasgi S, Surekha R, Koneru A, Manvikar V. A comparison of absorption inhibition and absorption elution methods for estimation of ABO blood groups in saliva. J Med RadiolPathol Surg. 2015 Jan;1(1):1-4.
[43]. Thaler R, Froum S, Chuba JV, Scopp IW. A quantitative study on the relationship of salivary blood group substances to periodontal disease. J Periodontal Res. 1976 Apr;11(2):116-20. PubMed PMID: 132517.
[44]. Graves MH, White JM, Fitzpatrick FA, Kuo MC. A comparison of absorption-inhibition and absorption-elution methods in the detection of ABO(H) antigens found in vaginal samples submitted in sexual offense cases. J Forensic Sci. 1978 Apr;23(2):345-52. PubMed PMID: 122745.
[45]. Kaur G, Sharma VK. Comparison of absorption-inhibition and absorption-elution methods in the detection of ABO (H) antigens in sweat stains. Current science. 1988 Nov 20:1221-3.
[46]. Nishimura A, Yamamoto Y, Nishi K. Expression of ABH and ABH-related antigen in secretory cells of indoor pets. Species analysis should be necessary prior to ABO blood grouping in stain analysis. Anil Aggrawal's Internet J. Forens Med. Toxcology. 2001 Jul;2(2).
[47]. Nishi K, Tanegashima A, Yamada M, IkeharaY, Yamamoto Y, et al. Lectin-and Immuno-Histochemistry on Mucous Substances of the Taste Buds and Lingual Glands in Some Mammals. In Advances in Forensic Haemogenetics 1994;(pp. 638-640). Springer, Berlin, Heidelberg.
[48]. Harrington JJ, Martin R, Kobilinsky L. Detection of hemagglutinins in dried saliva stains and their potential use in blood typing. J Forensic Sci. 1988 May;33(3):628-37. PubMed PMID: 3385376.
[49]. Thrumiaya T, Gayathri R, Priya VV. Efficacy and accuracy of ABO blood group determination from saliva. Journal of Advanced Pharmacy Education& Research| Apr-Jun. 2017;7(2).
[50]. Ruth MS, Purnadianti M, Marini MI. Blood group analysis from cigarette butts by absorption inhibition method: An experimental study. Journal of International Oral Health. 2020 May 1;12(3):275.
[51]. Nishi K, Rand S, Fechner G, Brinkmann B. Immunohistochemical localization of A, B, H, Le a and Le b antigens in human thymus using avidin–biotin complex technique. ActaCrimJpn. 1998;54:217-23.
[52]. Nishi K, Ito N, Hirota T, Fechner G, Rand S, Brinkmann B. Different expression of blood group A antigen in the mucous cells of salivary glands from Japanese and German nonsecretor individuals. Jpn J legal Med.1989; suppl. 220
[53]. Nishi K, Fukunaga T, Yamamoto Y, Yamada M, Kane M, Tanegashima A, et al. ABH-related antigens in human male genital tract. A histochemical examination. Int J Legal Med. 1992;105(2):75-80. PubMed PMID: 1520640.
[54]. Yada S, Ishimoto G, Okane M. Blood grouping an 88-years-old hair rope. Acta Crime Apon. 1968;34:90-2.
[55]. Miyasaka S, Yoshino M, Sato H, Miyake B, Seta S. The ABO blood grouping of a minute hair sample by the immunohistochemical technique. Forensic Sci Int. 1987 May-Jun;34(1-2):85-98. PubMed PMID: 3297953.
[56]. Smeets B, van de Voorde H, Hooft P. ABO bloodgrouping on tooth material. Forensic Sci Int. 1991 Sep;50(2):277-84. PubMed PMID: 1748363.
[57]. Neiders ME, Standish SM. Blood group determinations in forensic dentistry. Dent Clin North Am. 1977 Jan;21(1):99-111. PubMed PMID: 264468.
[58]. Suzuki K. Blood group determination in the tissues of human teeth. Jap J Legal Med. 1957 Mar;11:168-77.
[59]. Ramnarayan B, Manjunath M, Joshi AA. ABO blood grouping from hard and soft tissues of teeth by modified absorption-elution technique. J Forensic Dent Sci. 2013 Jan;5(1):28-34. PubMed PMID: 23960412.
[60]. Borgognini SM. New trends in blood group determination in human bones. Proc VIIth Intern CongrAnthropEthnolSci, Tokyo-Kyto. 1968 Sep;114.
[61]. Lee HC, Gaensslen RE, Pagliaro EM, Carroll-Reho J. ABH Typing of Human Bone. In 39th Annual Meeting, Am Acad Forensic Sci. San Diego, CA; 1987 Feb.
[62]. Saito Y, Kimura A, Sagawa K, Inoue H, Yasuda S, Nosaka M, et al. Analysis of blood group active glycolipids in hairs and nails, and development of a novel blood grouping method. J Wakayama Med Soc. 2003;54:21-9.
[63]. Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. Nature. 1985 Mar 7-13;314(6006):67-73. PubMed PMID: 3856104.
[64]. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 1995 Nov 11;23(21):4407-14. PubMed PMID: 7501463.
[65]. Liu WT, Marsh TL, Cheng H, Forney LJ. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol. 1997 Nov;63(11):4516-22. PubMed PMID: 9361437.
[66]. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring HarbSymp Quant Biol. 1986;51 Pt 1:263-73. PubMed PMID: 3472723.
[67]. McCord BR, Gauthier Q, Cho S, Roig MN, Gibson-Daw GC, Young B, et al. Forensic DNA Analysis. Anal Chem. 2019 Jan 2;91(1):673-688. PubMed PMID: 30485738.
[68]. Goodwin W, Linacre A, Hadi S. An introduction to forensic genetics. John Wiley & Sons. 2nd ed. 2011 Jun 28;53-62.
[69]. Oostdik K, Lenz K, Nye J, Schelling K, Yet D, Bruski S, et al. Developmental validation of the PowerPlex(®) Fusion System for analysis of casework and reference samples: A 24-locus multiplex for new database standards. Forensic SciInt Genet. 2014 Sep;12:69-76. PubMed PMID: 24905335.
[70]. Tautz D, Renz M. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. 1984 May 25;12(10):4127-38. PubMed PMID: 6328411.
[71]. Gettings KB, Kiesler KM, Faith SA, Montano E, Baker CH, Young BA, et al. Sequence variation of 22 autosomal STR loci detected by next generation sequencing. Forensic SciInt Genet. 2016 Mar;21:15-21. PubMed PMID: 26701720.
[72]. Allen M, Nilsson M, Havsjö M, Edwinsson L, Granemo J, Bjerke MH, et al. for simultaneous analysis of 10 STRs, 386 SNPs and the complete mtDNA genome. In Presentation at the 25th Congress of the International Society for Forensic Genetics 2013; 2-7.
[73]. Guo F, Yu J, Zhang L, Li J. Massively parallel sequencing of forensic STRs and SNPs using the Illumina® ForenSeq™ DNA Signature Prep Kit on the MiSeqFGx™ Forensic Genomics System. Forensic SciInt Genet. 2017 Nov;31:135-148. PubMed PMID: 28938154.
[74]. Moreno LI, Mills DK, Entry J, Sautter RT, Mathee K. Microbial metagenome profiling using amplicon length heterogeneity-polymerase chain reaction proves more effective than elemental analysis in discriminating soil specimens. J Forensic Sci. 2006 Nov;51(6):1315-22. PubMed PMID: 17199616.
[75]. Børsting C, Morling N. Next generation sequencing and its applications in forensic genetics. Forensic SciInt Genet. 2015 Sep;18:78-89. PubMed PMID: 25704953.
[76]. Tan E, Turingan RS, Hogan C, Vasantgadkar S, Palombo L, Schumm JW, et al. Fully integrated, fully automated generation of short tandem repeat profiles. Investig Genet. 2013 Aug 6;4(1):16. PubMed PMID: 23915594.
[77]. Williamson AL. Touch DNA: forensic collection and application to investigations. J Assoc Crime Scene Reconstr. 2012;18(1):1-5.
[78]. Minor J. Touch DNA: from the crime scene to the crime laboratory. Forensic Magazine. 2013 Apr;4.

[79]. Sowmyya T. Touch DNA: an investigative tool in forensic science. Int J Curr Res. 2016;8(02):26093-7.
[80]. Wickenheiser RA. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci. 2002 May;47(3):442-50. PubMed PMID: 12051321.
[81]. Pereira L, Alshamali F, Andreassen R, Ballard R, Chantratita W, Cho NS, et al. PopAffiliator: online calculator for individual affiliation to a major population group based on 17 autosomal short tandem repeat genotype profile. Int J Legal Med. 2011 Sep;125(5):629-36. PubMed PMID: 20552217.
[82]. Eduardoff M, Gross TE, Santos C, de la Puente M, Ballard D, Strobl C, et al. Inter-laboratory evaluation of the EUROFORGEN Global ancestry-informative SNP panel by massively parallel sequencing using the Ion PGM™. Forensic SciInt Genet. 2016 Jul;23:178-189. PubMed PMID: 27208666.
[83]. Meissner C, Ritz-Timme S. Molecular pathology and age estimation. Forensic Sci Int. 2010 Dec 15;203(1-3):34-43. PubMed PMID: 20702051.
[84]. Bauer CM, Niederstätter H, McGlynn G, Stadler H, Parson W. Comparison of morphological and molecular genetic sex-typing on mediaeval human skeletal remains. Forensic SciInt Genet. 2013 Dec;7(6):581-586. PubMed PMID: 23941903.
[85]. Vidaki A, Kayser M. Recent progress, methods and perspectives in forensic epigenetics. Forensic SciInt Genet. 2018 Nov;37:180-195. PubMed PMID: 30176440.
[86]. Horvath S, Zhang Y, Langfelder P, Kahn RS, Boks MP, van Eijk K, et al. Aging effects on DNA methylation modules in human brain and blood tissue. Genome Biol. 2012 Oct 3;13(10):R97. PubMed PMID: 23034122.
[87]. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 2014 Feb 3;15(2):R24. PubMed PMID: 24490752.
[88]. Garagnani P, Bacalini MG, Pirazzini C, Gori D, Giuliani C, Mari D, et al. Methylation of ELOVL2 gene as a new epigenetic marker of age. Aging Cell. 2012 Dec;11(6):1132-4. PubMed PMID: 23061750.
[89]. Pan F, Chiu CH, Pulapura S, Mehan MR, Nunez-Iglesias J, Zhang K, et al. Gene Aging Nexus: a web database and data mining platform for microarray data on aging. Nucleic Acids Res. 2007 Jan;35(Database issue):D756-9. PubMed PMID: 17090592.
[90]. Freire-Aradas A, Phillips C, Lareu MV. Forensic individual age estimation with DNA: From initial approaches to methylation tests. Forensic Sci Rev. 2017 Jul;29(2):121-144. PubMed PMID: 28691915.