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REVIEW

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A glance on Immunogenetics Laboratory: from the origins to the future

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Abstract

Histocompatibility and Immunogenetics (H&I) laboratories have currently a significant relevance in clinical and research medical fields. The purpose of this review is to investigate their role through an *excursus* between bioethics, histocompatibility history and laboratory organization. The histocompatibility laboratories play an essential role in the transplantation process, and, through their molecular techniques, they can affect clinical decisions in a remarkable way. Half a century has passed from the first paper, published in 1958, to the modern deep sequencing techniques; in these years through specific guidelines and international standards drafted by 2 specific bodies (ASHI and EFI), H&I laboratories are subjected to continuous controls by inspection authorities formed by professionals in the Immunogenetics field. For their functioning, H&I laboratories require: a structure and devices, a dedicated room and a clear path to samples workflow. In these laboratories, the personnel must be specialized even just in a single precise assignment, and every member is assigned to a role according to the experience matured over the years. In these laboratories, the role of Director/Co-Director or Technical Supervisor is usually assigned to a staff member with a minimum of 4 years of experience in Immunogenetics or transplantation fields, following the EFI/ASHI Standards. Bioethics is another important aspect because, in the last few years, there has been a major change in legal regulations on informed consent. The advent of digitization has pushed many laws on personal and genetic data treatment to be adapted to most modern guidelines, although they may differ according to the countries in Europe and USA. In the last years, the H&I laboratories turned as great resources with many clinical features and nowadays they may lead an important transformation in research and clinical fields.

Keywords HLA, Immunogenetics, Bioethics, Informed consent, Immunogenetics laboratory, Accreditation programme, Laboratory organization

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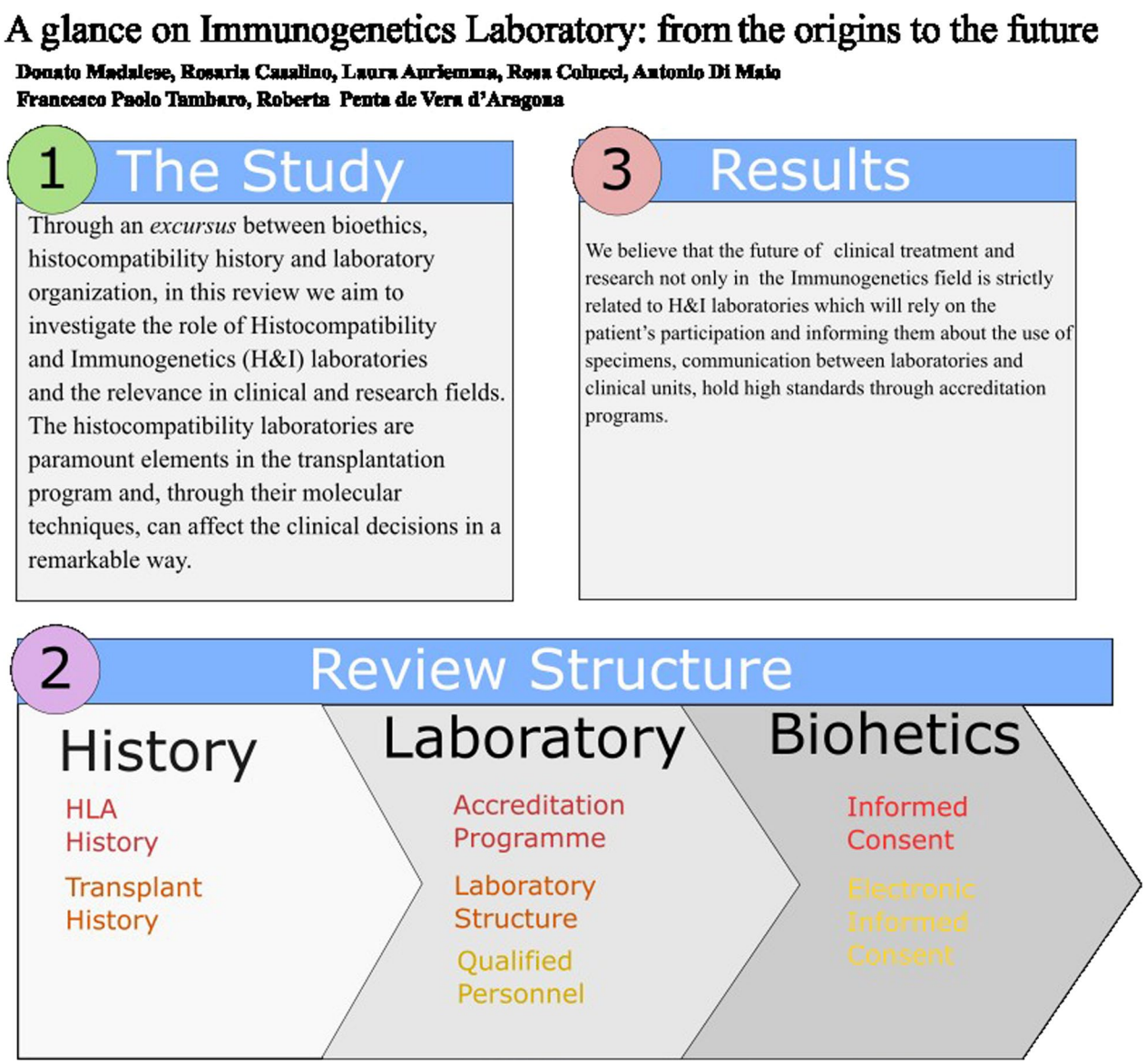
Graphical abstract



Introduction/background

Immunogenetics and Human Leukocyte Antigen (HLA) typing are powerful tools to investigate several Autoimmune Diseases (ADs) and histocompatibility transplantation. In the last years, there has been a great technological revolution in DNA sequencing and the resolution capacities of different devices. A few years have passed from serological typing to molecular typing through Sequencing and Next-Generation Sequencing (NGS). This resulted in great benefits in achieving higher allelic resolution and lower ambiguity at a comparable cost, to Sequence-Specific Oligonucleotides (SSO)/Primers sequence-specific (SSP) [1]. Inaccuracies in HLA typing can generate higher risk of transplant rejection for the patient involved [2]. The daily management of H&I laboratory requires the presence of highly qualified personnel, compliance with specific standards and high knowledge in the clinic field, and also of bioethics field. In this article, we will demonstrate how immunogenetics is not only HLA typing but also a very modern highly regulated tool.

Main text

Immunogenetics history

HLA origins

In 1958 was published the first paper which laid the foundation of the HLA complex. These described antibodies in human sera from multi-transfused patients or multiparous women that agglutinate their own white cells [3, 4]. Jean Dausset, a French hematologist author of these papers, tested sera from seven different patients who received multiple blood transfusions for neutropenia. None of those sera agglutinated white cells from their respective (autologous) donors. However, all seven sera contained antibodies that agglutinated leukocytes from 11/19 non-self (allogeneic) subjects. Dausset named the antigen agglutinated by those sera as *MAC* in honor of three individuals who had been important volunteers for his experiments and whose names began with the initials M, A and C, respectively [3, 4]. This HLA antigen, named MAC, is today molecularly referred to as HLA-A*02 [4].

In 1962, Jon J. van Rood analyzed the reaction patterns of 60 sera from multiparous women against leukocytes from a panel of 100 donors. He found that some sera sample detected a diallelic system of leukocyte antigens, which he called 4a and 4b (today named HLA-Bw4 and -Bw6, respectively) [4].

The first real task force dedicated to studying the mystery of HLA was in 1964 at Duke University, Durham, NC. Here, the first of three International Histocompatibility Workshops (IHWSs) was established where researchers in this field may meet in order to compare their up-to-date techniques and last results [4, 5]. In that case, twenty-three researchers tested the same sera and cells with their own techniques such as leuko-agglutination, the indirect antiglobulin consumption test, mixed agglutination, and complement fixation; most of the results were discordant. Thus, in 1965 (second IHWS), researchers from 14 different groups tested their own antisera versus 45 common panel cells. It was found that several local specificities were identical or almost identical [4, 6].

In 1967, the third IHWS was hosted by the University of Turin, Italy, and organized by the geneticist Ruggero Cappellini. The main aim was to study the genetics of the hitherto identified leukocyte antigens. Blood from 11 families, including monozygotic twins, were tested 'blindly' by the different researchers with their own typing antisera and techniques. Several researchers were now using the quicker and more reliable micro-cytotoxicity test, initially developed by Terasaki [4], which later became the standard serological typing technique for HLA antigens. In 1968, an HLA Nomenclature Committee was established sponsored by World Health Organization (WHO). In 1980, Dausset received the Nobel Prize, shared with Snell and Baruch Benacerraf.

Transplant history

In the History, the First transplant reported dates back in 1817, when a French physician, Henri Dutrochet, wrote a letter to the *Gazette de Santé* reporting a skin transplant based on a story from his brother-in-law, an officer stationed in India. In this letter is described that a soldier, who was under his command, had his nose amputated as a form of punishment. So, he sought among the local people for a skilled man in surgery for the reconstruction of the nose [7].

While there is uncertainty regarding the veracity of Dutrochet's letter, the first verifiable document on transplantation has been reported in 1869. Jacques-Louis Reverdin, a Swiss surgeon, used small samples of epidermis-pinched layers of skin, successfully demonstrated skin graft, and until World War I (WWI), this technique was used in burn treatment. After WWI and until World War II (WWII), this technique was replaced with the use of tannic acid; however, but it was soon realized that this procedure caused liver necrosis. Thus, grafting again became the gold standard [7].

Since 1950, there was great interest in organ transplants, and in 1959, the first kidney transplant was performed at Charity Hospital in Louisiana. In 1967, Dr. Thomas Starzl performed the first liver transplant on a human at the University of Colorado and the first three patients survived between 7 and 22 days. From this date onward, research continued and led to the discovery of cyclosporine in late 1970 [7].

Laboratory organization

Infrastructures

In order to proper work, H&I laboratory need important structurally separated work areas following a definite accreditation plan. Immunogenetics laboratory needs well-defined rooms with specific equipment that cannot be moved from one area to another [8] (Table 1).

The first room is called Pre-PCR or Pre-Amplification room; here, the sample is accepted, DNA extraction is performed and the first step of every HLA technique is started. In the Pre-PCR room, there should be a refrigerator, a freezer, laboratory bench, a nucleic acid extractor, a laminar flow hood, centrifuges/micro-centrifuges and a dedicate laboratory cabinet.

Refrigerator must be at + 4 °C with well-ordered shelves and well separated according to sample type/source, DNA samples, reagents/kits IVD and reagents/kit RUO. Every shelf must be properly labelled with the category name of which that it contains.

Table 1 Organizational model of the H&I laboratory

Room	Equipment	Shelves organization
PRE-PCR	Refrigerator + 4 °C	1. Blood samples or other sources 2. DNA sample 3. Kit IVD 4. Kit RUO
	Freezer – 30/– 40 °C	Shelf → rack → box
	Laboratory bench	
Filter Area	PCR Amplifier/ nothing	
	Undress cabinet	
POST-PCR	Refrigerator + 4 °C	
	Freezer – 25/– 30 °C	
	Molecular typing equipment	

The division into three areas is necessary to prevent DNA contamination and perform PCR safely

The freezer temperature must be at − 30 °C or lower, in order to proper stock the DNA/Buffy coat samples and most of the reagents daily used in HLA. Every shelf must contain boxes organized in racks, and each rack must contain only one sample type. On average, each rack contains about 9 boxes and each box must be labelled with the category name of what it contains and the ID of samples included.

A second room linked to Pre-PCR is named "Filter Area". This room could contain PCR amplifiers or nothing. Usually, it is an empty room used to separate the Pre- from the Post-zone and where the operators can undress from Pre- clothing.

The last room is named Post-PCR or Post-Amplification room. Here, the amplified plates are processed with the routine methods of the HLA laboratory. In this room is necessary the presence of a freezer and refrigerator for reagents, where momentarily stock the amplified plates, chemical hood, Sequencer, SSO equipment, laboratory bench, centrifuges/micro-centrifuges, SSP electrophoresis equipment, and NGS sequencer.

Quality assurance

Each Immunogenetics Laboratory attends a detailed quality schedule and well-defined standards. Currently, there are 2 institutions that draw up the standards for the Immunogenetics laboratories: European Federation for Immunogenetics (EFI) and the American Society for Histocompatibility and Immunogenetics (ASHI).

Through these two institutions and their guidelines, the Immunogenetics Laboratories work under rigorous standards. First of all, qualified staff is required such as a quality manager, qualification methods and well-defined parameters to perform analysis, specific documentations about each process in the laboratory.

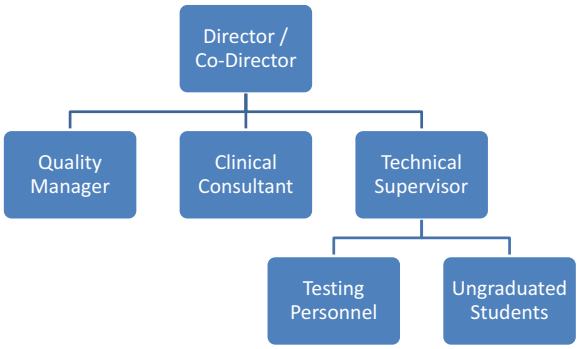


Fig. 1 Hypothetical Organization Chart

The first Immunogenetics laboratories achieved EFI accreditations in 1995 and, currently, there are over 260 EFI-accredited laboratories in 36 different countries [9].

The purpose of accreditation programme entity is to certify that a laboratory meets the requirements of standards and the level of assurance, that the service provided, is appropriate to the quality standards. Normally, the generic medical laboratory can be accredited through a generic set of standards such as ISO 9001:2015 or ISO 15189:2022 which is specific for medical laboratories. For Immunogenetics Laboratories, there are also specific accreditation programmes organized by the professional Accreditation Committee of ASHI or EFI. They use a specific standard that is regularly reviewed and updated taking into account the most recent clinical evidence in histocompatibility, Immunogenetics and transplantation fields.

Each laboratory that voluntary wants participate to in accreditation programmes will receive an inspective visit from 3 of 100 voluntary inspectors, professionals who work in an EFI-accredited laboratory and with many years of experience [9].

Qualified personnel

As mentioned, Immunogenetics and Histocompatibility are medical fields that require specialized personnel with a tight training programme. This training programme is followed in an accredited laboratory and lasts a minimum of three months [10, 11] (Fig. 1). The titles may change by state and country, but we may classify as follows:

- *Director / co-director*: usually a PhD in chemistry, biology, physic or in medicine. Hold a minimum of 4 years of experience at full time in HLA and Immunogenetics.
- *Clinical consultant*: Medical doctor that provides consultation about the clinical interpretation of

test results, and ensures an appropriate report with patient information.

- *Technical supervisor*: a PhD in chemistry, biology, physic or in medicine. He must have a minimum of 2 years of experience in HLA and Immunogenetics, and he is responsible for the technical and scientific oversight of the laboratory
- *Quality manager* (QM): who must establish and maintain a comprehensive quality management programme covering all aspects of the accredited facility addressed by these standards

Testing personnel: a PhD or master's or bachelor's degree in chemistry, biology, physic or in medicine. This figure professional is responsible for specimen processing and test performance, following the Technical supervisor's instruction.

(a) Director/co-director

The *director* is led by a Management Committee and must have documentation about the professional competence in the appropriate activities in which the laboratory is engaged and a minimum of 2 years of experience. The principal function of the Director is the daily managing of the laboratory, including the personnel recruitment, test results recording and reporting, and assure that the environmental conditions are appropriate for tests performed and safe from physical, chemical and biological agents. Furthermore, the director must guarantee that the test procedures used are adequate and that laboratory personnel are performing the methods according to standards. Finally, the director verifies that all documentation complies with the standards used and that all personnel are constantly updated and actively take part in training courses in histocompatibility to improve their skills [10, 11].

(b) Quality manager

The q*uality manager* (QM) is another figure designated by the Director and can overlap with other figures present in the laboratory. QM must establish and follow written policies and procedures to assess and document the competency of staff, their proficiency evaluation, testing performance and, write a manual procedure about all tests and assays performed in the laboratory. All manual procedure cannot be replaced by the manufacturer's instruction or operator manual. Every procedure must be reviewed and updated as required by Standards.

Work flow

Sample collection

The first crucial step in DNA analysis is the sample collection (Fig. 2). Isolation of DNA from some samples in adequate quantities is an important phase in genetic analysis and research fields. Nowadays many kits allow the DNA extraction from different sources such as

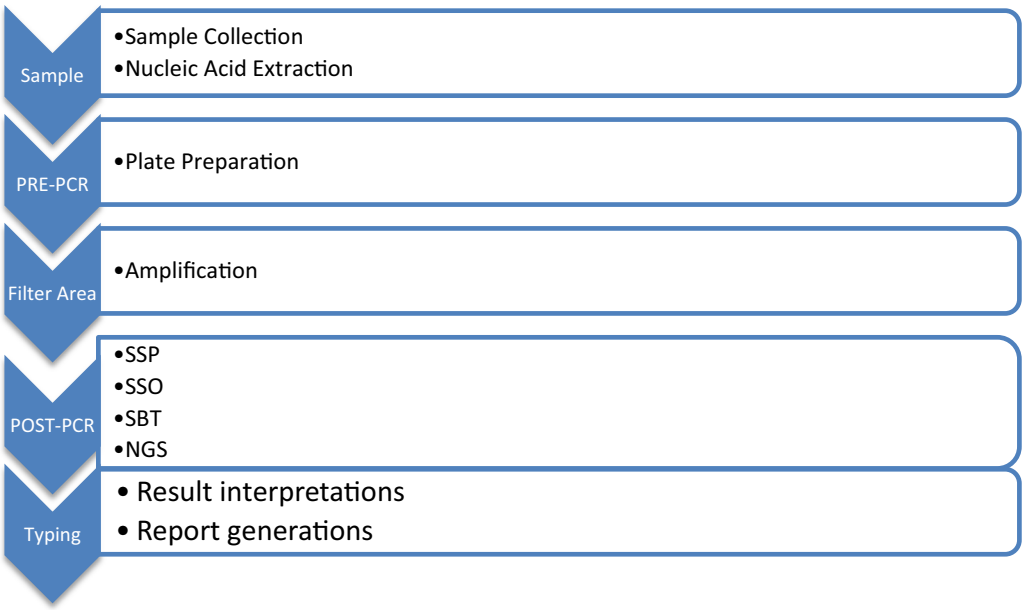


Fig. 2 Flowchart of work flow. *SSP* sequence-specific primer, *SSO* sequence-specific oligonucleotide probe, *SBT* sequence-based typing, *NGS* next-generation sequencing

human hair, blood, saliva, buccal swab, body fluids and other. Blood is the best source for DNA extraction for its low invasiveness and nucleated cellular yields.

Correct patient identification is a crucial step for safe care, and it is a primary goal of any healthcare organization. Indeed, identification errors are major problem in healthcare [12]. In Table 2 below reported the action to patient identification.

Blood specimens are obtained from the patients/donors by venipuncture and is performed by nurses personnel following WHO Guidelines [13]. Blood sample is collected in two (2) EDTA tube [14, 15].

Some patients may have intravenous catheters and it may lead to a significantly higher risk of hemolysis compared with standard blood collections using straight needles [16].

Nucleic acid extraction

In order to perform a Polymerase Chain Reaction (PCR) and molecular typing, the first operational step is Nucleic Acid Extraction (NAE). The gold standard source to perform NAE is whole blood or buffy coat and there are different techniques to perform NAE with different yields.

The most common method to perform this operation is the Phenol–Chloroform Extraction. It is widely used in different laboratories and it consists in mixing the sample with two organic solvents (Phenol and Chloroform) and then centrifuge the mix. At the end of centrifugation, the aqueous phase containing DNA is separated from the lower phase containing proteins and other. After washing with 70% ethanol, DNA is collected in TE buffer or distilled water [17].

Modern techniques of NAE are based on magnetic beads. This method is quite trivial and commonly used in automated extraction technologies. The beads are charged negative, and this allow them to bind protein and other cellular debris on their surface in a solid–liquid extraction; thus, DNA can be isolated by simply removing the beads from the solution [17].

Table 2 How to perform a sample collection

Labeling	Report on Tube: Name, Surname Date of Birth Date of collection Department/External Structure Progressive Identification Number (ID) Labeling collection tube before blood collection
Confirmation questions	What is your Name? When are you born? What is your clinical diagnostic bias?
Signature	Collector nurse signature on each collection tube

From identification to labeling tubes

The quantification of the Nucleic Acid extracted may be performed through spectrophotometry technology such as Nanodrop or through fluorometry [18, 19].

Typing

(a) Serological typing

HLA Typing is performed through various tests that are quite similar in many HLA laboratories in the world. The first HLA typing techniques was serum-based and it has been used as a standard procedure, for several years before molecular typing. Through the complement-mediated microlymphocytotoxicity technique, it was possible typing HLA class I and II antigens into serum [20]. Indeed, peripheral blood lymphocytes (PBLs) express HLA-I antigens and are used for the serologic typing of HLA-A, HLA-B, and HLA-C. HLA-II typing is done with B lymphocytes isolated from PBLs because these cells express class II molecules [21]. Serological typing is performed in multi-well plastic trays in which each well contains a serum of known HLA specificity. Lymphocytes are plated, incubated, and then complement (rabbit serum) is added to mediate the lysis of antibody-bound lymphocytes [22].

(b) Molecular typing

Unfortunately, many serological variants or HLA-I/II were serologically indistinguishable because these variants are different from wild type by few amino acids [22]. Despite all, these little changes can be relevant in transplant field. PCR is the technology used for HLA molecular typing and currently four different methods for HLA molecular typing exist.

The first of them is named Sequence-Specific Primer (SSP), in which for each Locus (Low Resolution: Locus A, B, C, DRB1, etc.) or specific allele (High Resolution: A*02, B*51, C*04, DRB1*11, etc.) exist a plastic plate with different number of wells. Each well has a specific primer that binds the DNA sample if Single Nucleotide Polymorphism (SNP) of the allele is present. After PCR amplification, amplified DNA in each well is dispensed into 2% agarose gel prepared with Tris–Acetate-EDTA/Tris–Borate-EDTA (TAE/TBE) buffer and a DNA dye. The percentage of agarose used varies depending on the laboratory's requirements and the technique being employed. In SSP method, a concentration of 2% agarose is ideal because the resulting mesh structure allows for optimal separation of even small fragments, as short as 80 base pairs [23, 24]. The Ladder, a molecular-specific weight used to define the weight sample lanes, is added into another gel well. The choice between TAE or TBE

depends on the costs, the size of the nucleic acid fragments that have to migrate and the pH [25]. Instead, the choice of DNA dye depends on the toxicity (mutagenic) and excitation wavelength (nm) [26]; gold standard dye is ethidium bromide, a strong DNA intercalator that binds between DNA base pairs [27]. Thus, researchers have developed new non-mutagenic and low cost DNA dyes, such as GreenSafe DNA Gel Stain (Canvax Reagents SL, Valladolid, Spain), that are able of generating similar results to ethidium bromide dye without the toxicity side-effect [26]. Afterward electrophoresis, agarose gel is transferred on transilluminator system with different lamps according to the wavelength necessary to excite the dye used. A digital camera is used to acquire the gel image, and it is analyzed on a computer with dedicated software and an interpretation table.

The second important molecular typing technique is the Sequence-Specific Oligonucleotide Probe (SSO/SSOP). This technique combines the PCR technology and sequence-specific hybridization probe and was applied for the first time with HLA-II loci due to limitations of serological techniques on DR locus typing [28]. The method is based on a locus-specific amplification probe. After the first PCR, each amplified DNA is hybridized with sequence-specific oligonucleotide probes. These probes are usually immobilized on a solid support like microsphere and linked with fluorochromes (Phycoerythrin-PE). The hybridized DNAs are acquired on specific instruments like Luminex (Luminex Corporation, Austin) and data is analyzed on associated software like Fusion (LABType, One Lambda, Inc.).

Sequence-based typing (SBT) has been the best method for HLA typing over the past decade. It was developed by Frederick Sanger in 1977 and is based on chain termination [29]. This method is based on the use of Dideoxynucleotides (ddNTP) instead of deoxynucleotides (dNTP). The ddNTP lacks the 3′ hydroxyl group that is required for the extension of DNA chains, thus cannot bind the 5′s phosphate of the next dNTP. When DNA polymerase protein interact with a ddNTP, the chain extension is blocked and ddNTP emits a signal in the fluorescence range. Each ddNTP (ddATP, ddCTP, ddGTP, ddTTP) is linked to different markers and emit different signal at different fluorescence wavelength [29, 30]. Then, the capillary electrophoresis is performed and a specific software makes DNA sequencing. In the end, the sequencing result is analyzed through software and, in according to Common and Well Documented (CWD) HLA, it is compared to the HLA database to complete the DNA typing.

The last and most modern technique we would like to describe here is the Next-Generation Sequencing (NGS, indeed It has mostly superseded conventional Sanger sequencing, despite that NGS is routinely used in the research field and not yet widely applied in clinical practices [31]. Nowadays, exist different techniques to perform the NGS method, for example: Illumina, SOLiD (ABI), PacBio, and Oxford Nanopore [32]; the most commonly used in the HLA field is based on Solexa-Illumina technology [29]. The first step to performing these techniques is "Library Construction" which is the preparation and fragmentation of nucleic acid the target into a form and length compatible with the choice method. This step is performed through a physical method (sonication) or an enzymatic method (endonuclease). Library size is dictated by the sequencing application [33]. Later this step, following different protocols, specific primers are linked on a specific support (such example a glass support named "flow cell" in illumine technology). Every DNA fragment is then amplified using nucleotides linked to specific fluorochromes [34, 35].

In the state of art of high-throughput sequencing technologies, commonly known as NGS, both Long Read Sequencing (LRS) and Short Read Sequencing (SRS) are utilized as sequencing methodologies.

LRS offers several advantages; in fact, LRS provides the ability to sequence longer DNA fragments allowing the detection of large structural variations and complex genomic rearrangements. This is particularly useful in HLA typing, because it enables the identification of novel alleles, and accurately resolving highly polymorphic regions. In addition, LRS can provide phasing information allowing the determination of haplotypes; which is important in revealing genetic diversity and disease associations. Furthermore, the LRS platforms have improved more and more over time, reducing error rates and improving sequencing accuracy. However, LRS also has some limitations as the higher error rate compared to Short Read Sequencing. This is related to the difficult in accurately reading long DNA fragments and the error-prone nature of certain LRS technologies. Furthermore, the LRS per sequenced base is usually higher expensive compared to SRS because longer sequencing reading requires higher computational resources for data analysis and storage [36–38].

SRS has its own set of advantages. First of all, SRS technologies, such as Illumina, are highly accurate providing high-quality sequencing data. They are well-established and widely used in many research and clinical settings. SRS also has a lower cost per sequenced base compared to LRS, making it more cost-effective for large-scale sequencing projects. Moreover, the shorter read lengths of SRS can be advantageous for applications, such as targeted sequencing where high coverage is required. Nevertheless, SRS has some limitations to consider at the time to select the most suitable for the application desired. The shorter read lengths may limit the capacity

to correct resolve highly polymorphic regions or detect complex structural variations. In this sense, phasing information may be challenging to obtain only through SRS affecting the haplotype determination accuracy. Finally, the sequencing repetitive regions of the genome could be more difficult with the SRS platforms, leading to potential gaps or ambiguities in the data [36, 37].

Bioethics

After the WWII, in 1947, a series of ethical principles for human experimentation have been established drafting a series of regulations that took the name of "Nuremberg code". This treaty contains all the indications for carrying out research and experimentation while maintaining ethics and without harming the individual [39]. However, the first attention on ethical problems has been reported by Henry Beecher, a professor of anesthesiology at Harvard Medical School that in 1966 focused his attention to the failure to inform patients about the risks involved in experimental treatments [40].

In 1969, the Hastings Center/Institute of Society was founded, Ethics and the Life Sciences developed a modern health care ethics named bioethics. The Hasting Center lead methods and intellectual standards of bioethics through its own journal "The Hasting Center Report" [40].

Bioethics for Immunogenetics and research is an intense and growing field of interest; here, we present a brief overview of Informed Consent (IC) and how IC is managed around the world.

Informed consent

IC is a critical element of the research field, clinical studies and genetic data. The European Parliament and the Council of the European Union define genetic data as "personal data relating to the inherited or acquired genetic characteristics of a natural person which result from the analysis of a biological sample from the natural person in question, in particular chromosomal, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) analysis, or from the analysis of another element enabling equivalent information to be obtained." [41]. The Guideline for Good Clinical Practice (GCP) defines IC as a document in which a subject voluntarily confirms his/her willingness to participate in a particular study, after having been informed of all aspects of the study that are relevant to the decision of the subject to participate. IC is a form paper-based and serves as a legal ground for processing personal data according to the basic principles embedded in General Data Protection Regulation (GDPR) [42]. The patient must be capable to take an unforced decision about whether to undergo the procedure or intervention, and the IC is an assessment of the patient's understanding. IC is different for each application field and must ensure:

- Treatment of sample
  - If samples will be used for research or diagnostic purpose
  - Use of the samples for the stated purpose only
  - If samples will be stored or discarded
- Personal data
  - If samples will be anonymized
  - Who/where personal data will be recorded
  - Who will be able to collect the diagnostic results

Electronic informed consent

Electronic Informed Consent (eIC) is a digitalized IC form. At the moment, there is no shared guideline about its regulation between the USA and the single members of the European Union (Table 3). Indeed in USA, there are several sector-specific privacy and data security laws at federal, state, and local levels like Health Insurance Portability and Accountability Act (HIPAA - 1996) and in 2016, FDA and HHS, issued guidance on eIC for the Clinical Study field [43]. Instead in Europe, there is a lack of uniformity among member states, some of these allow eIC with or without explicit regulatory bodies, and other not allowed it [42]. The Swiss Ethics Committees recommended that the researchers, sponsor, project leaders or other, have to discuss plans for using an eIC with them, and that the hand-written signature of participants is mandatory in the document for their consent (eIC is not allowed). Instead in Finland, the National Committee on Medical Research Ethics (TUKIJA) accept the eIC, this despite not being mentioned in the national legislation (eIC is allowed but not regulated). The last is the Austrian case. Article 4 of the Federal Act on Electronic Signatures and Trust Services for Electronic Transactions describes

Table 3 Summary table of states that allowed or not eIC [42, 44]

EU States			USA
Allowed		Not allowed	Allowed
Regulated	Not regulated	Regulated	Regulated
Austria	Finland	Hungary	
Belgium	Italy	Switzerland	
Germany	Portugal		
Netherlands	Spain		
United Kingdom			

that electronic signature satisfies the legal requirement of written form and the Austrian Federal Office for Safety in HealthCare specific that electronic information such as audio and video can be used as informed consent [42].

Conclusions

Immunogenetics, histocompatibility and transplantation fields are rapidly changing areas of science and medicine. The modern deep sequencing methods allow not only HLA typing but also the research of other genetic systems which are increasingly being recognized as relevant to the immune response like KIR, MICA/MICB, HLA-E and HLA-G.

In conclusion, we may consider the following: DNA sequencing has undergone significant advancements over the years. NGS has largely replaced the traditional Sanger sequencing and has become, at the moment, a routine tool in research; although its clinical adoption is still quite limited. Among the different NGS technologies available, Illumina based on Solexa-Illumina technology is the most commonly used in the field of HLA typing.

The LRS offers several advantages, including the ability to sequence longer DNA fragments, enabling the detection of large structural variations and complex genomic rearrangements. This is particularly valuable for HLA typing as it allows the identification of novel alleles and accurate resolution of highly polymorphic regions. Additionally, LRS provides the phasing information, facilitating haplotype determination, which is crucial for genetic diversity understanding and disease associations. Over time, LRS platforms have improved, reducing error rates and enhancing sequencing accuracy. However, LRS has demonstrated some limitations, such as higher error rates compared to SRS and a higher cost per sequenced base. Furthermore, longer sequencing reads require more computational resources for data analysis and storage. In comparison, SRS has shown its own advantages. In fact SRS technologies, like Illumina, are highly accurate and provides high-quality sequencing data. They are widely used in research and clinical settings and have a lower cost per sequenced base compared to LRS, making them more cost-effective for large-scale sequencing projects. Additionally, the shorter read lengths of SRS can be advantageous for certain applications, such as targeted sequencing that requires high coverage. However, SRS has also limitations to be considered, including the limited ability to resolve highly polymorphic regions or detect complex structural variations. Obtaining phasing information with SRS only it is not trivial, this affecting the accuracy of haplotype determination. SRS platforms may also have difficulties in sequencing repetitive regions of the genome, resulting in potential gaps or ambiguities in the data.

Therefore, at the time to choose the sequencing method for HLA typing most suitable for their purposes, researchers and clinicians should consider the specific requirements of their study or clinical scenario. For efficient HLA family screening and disease typing, we believe that the combination of SSO and SRS-NGS methods is the ideal approach, with the addition of SSP/SBT and NGS-LRS as supplementary or research-focused tools. This combination increases efficiency and accuracy.

H&I laboratories play a crucial role in the future of clinical treatment and research, relying on patient participation and ensuring proper communication between laboratories and clinical units, while maintaining high standards through accreditation programmes.

We believe that the future of immunogenetics research and clinical treatment is closely linked to H&I laboratories; which will rely on active patient participation, informing them about the use of specimens, and communication between laboratories and clinical units; this last maintaining high standards via accreditation programmes.

Abbreviations

AD	Autoimmune disease
CWD	Common and well documented
EDTA	Ethylenediaminetetraacetic acid
eIC	Electronic informed consent
FDA	Food and Drug Administration
GCP	Good clinical practice
GDPR	General data protection regulation
H&I	Histocompatibility and immunogenetic
HHS	Department of health and human services
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
IC	Informed consent
ID	Identification number
IHWS	International histocompatibility workshop
IVD	In Vitro diagnostic
LRS	Long read sequencing
MHC	Major histocompatibility complex
NAE	Nucleic acid extraction
PBL	Peripheral blood lymphocyte
PCR	Polymerase chain reaction
PE	Phycoerythrin
QM	Quality manager
RUO	Research use only
SRS	Short read sequencing
SSP	Sequence-specific primer
TAE	Tris–acetate-EDTA
TBE	Tris–borate-EDTA
WHO	World Health Organization

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Author contributions

DM contributed to the study conception and design. RP and FPT supervised the manuscript. ADM read and reviewed the manuscript. All authors read and approved the final manuscript. All authors have approved the manuscript for submission.

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Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethic approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
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