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Methods of identification of human immunodeficiency virus (HIV) infections in Polish blood donors (2005–2022)

Ewa Sulkowska, Piotr Grabarczyk

Department of Virology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Summary

The human immunodeficiency virus (HIV) attacks the immune system to cause a lifelong severe illness called AIDS (acquired immunodeficiency syndrome). The HIV infection is a serious condition associated with high costs of therapy and care. Since the beginning of the AIDS/HIV epidemic the number of HIV infected people worldwide has exceeded 84 million, and about 40 million have already died of AIDS.

Transfusion of blood and blood components is one of the transmission routes for HIV infection. The risk of transmission of infection by this route has been significantly reduced e.g. by introducing mandatory screening tests for blood donors. There are still however reports of sporadic cases of transfusion transmitted infections related to the so-called diagnostic window and virus polymorphism.

In Poland, mandatory testing of anti-HIV were introduced for all blood donors in 1987, and HIV RNA in 2005. Enzyme immunoassays (EIA) have gradually been improved and currently all Polish Blood Transfusion Centers (BTCs) analyze both IgG and IgM antibodies as well as p24 antigen (IV generation tests) in donors. HIV RNA is tested in individual donations (IDT) or in mini pools (MP); the sensitivity expressed as 95% limit of detection [95% LOD] is 45–18 IU/mL and 1469 IU/mL — 302 IU/mL, respectively.

Verification tests were always performed at the Department of Virology of the Institute of Hematology and Transfusion Medicine (IHTM) with Western blot (WB) and molecular biology methods: transcription mediated amplification (TMA) and real-time polymerase chain reaction (RT-PCR).

This publication presents detailed strategy and methodology for conducting screening and verification tests for HIV in Polish blood donors in the years 2005–2022. The study is an extension and update of a part of the author's doctoral dissertation and initiates a discussion on the donor qualification process and the effectiveness of screening studies.

Key words: RNA HIV; anti-HIV; p24 antigen; blood donor; screening tests; NAT; WB HIV

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Correspondence address: dr n. med. Ewa Sulkowska, Department of Virology, Institute of Hematology and Transfusion Medicine, ul. Chocimska 5, 00–791 Warszawa, Poland, e-mail: esulkowska@ihit.waw.pl
Translation: mgr Krystyna Dudziak

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Introduction

More than 30 years after the discovery of the HIV virus, the epidemiology of HIV infections worldwide is still a significant public health care challenge. Since the beginning of the AIDS epidemic, approximately 85 million people have been diagnosed with HIV and about 40 million have died [1]. In Poland, since the beginning of the epidemic (1985), the infection has been detected in about 28,000 people. AIDS was diagnosed in nearly 4 thousand people and 1,448 patients have died [2].

Currently, transfusion of blood and blood components is safer than ever before. Procedures for reducing the risk of pathogen transmission (HIV included), have been successively implemented in the Polish blood transfusion service as reflected in published documents and legal acts [3–8] pursuant to the recommendations of European Union directives [9–12] and the World Health Organization (WHO) [13]. Manufacturers of equipment and tests have Food and Drug Administration (FDA) certificates [14]. However, despite implementation of all procedures strengthening the safety of blood and blood components, sporadic cases of transfusion transmitted HIV infections are still reported worldwide [15–19].

Discovery of HIV vs transfusion safety; beginnings of testing in transfusion medicine

AIDS (acquired immunodeficiency syndrome) was first recognized in the summer of 1981 in the United States. Young homosexual men developed opportunistic infections which resulted in severe pneumonia caused by *Pneumocystis carinii* bacteria. Patients were emaciated with facial lesions caused by a rare type of cancer called Kaposi's sarcoma. The characteristic feature of the disease was lymphopenia and a significant decrease in the CD4+ lymphocyte count [20]. Observations and epidemiological data from 1982 showed that the newly described syndrome spread to other groups of patients including hemophiliacs and drug addicts and manifested properties of infectious disease (transmitted through body fluids, infected blood or blood products) [21–26]. In September 1982, the CDC (Centre for Disease Prevention and Control) suggested AIDS as the name for the described disease [20]. In early 1983, AIDS was first reported among women-partners of infected men [24]. There have also been reports of AIDS in children who had most likely contracted the disease during pregnancy, labor or soon after birth [27, 28].

In March 1983, recommendations for AIDS prevention were issued in the United States of America and individuals from high risk groups were deferred/discouraged from donating blood and blood components for clinical use. The aim was to reduce the risk of AIDS transmission through transfusion of blood or blood components. At that time, transfusion-related AIDS accounted for approximately 2% of all AIDS cases reported in the United States [29]. At the beginning of the epidemic it was estimated that as many as 1 per 100 donations could have been responsible for HIV or HCV infection [30]. The March recommendations had no significant impact on the procedures related to the safety of blood and blood components because the proposed donor eligibility/qualification strategies in the USA were questioned as ineffective or discriminatory towards the homosexual male community [31].

Further studies on identification of the cause of AIDS infections led to the isolation of a new virus at the Pasteur Institute in Paris in May 1983. The virus was named LAV (Lymphadenopathy-Associated Virus), later LAV_{BRO} (BRO — from the first letters of the patient's last name). This virus was signaled as the hypothetical cause of AIDS [32]. The newly discovered retrovirus had many of the characteristics of the previously discovered human T-cell leukemia virus (HTLV). The virus was isolated from the RNA genetic material of a Caucasian patient with AIDS. Ultimately, the newly identified virus was classified as human T-lymphotropic retrovirus [32].

The next step towards identifying the factors that cause AIDS was the discovery of American scientists from the National Cancer Institute in Bethesda. In April 1984, the Institute announced that AIDS was caused by HTLV-III retrovirus. During a joint conference with the Pasteur Institute, LAV and HTLV-III were announced as identical and were the probable cause of AIDS [33]. At that stage it was assumed that a vaccine could become available within two years [34]. In 1986, the newly discovered virus was named HIV [35]. There were numerous controversies as to which center was the first to isolate HIV. The dispute seems to have been settled by the Nobel Prize awarded in Medicine and Physiology in 2008 which went to Professors Françoise Barré-Sinoussi and Luc Montagnier for the discovery of the human immunodeficiency virus (HIV) [36].

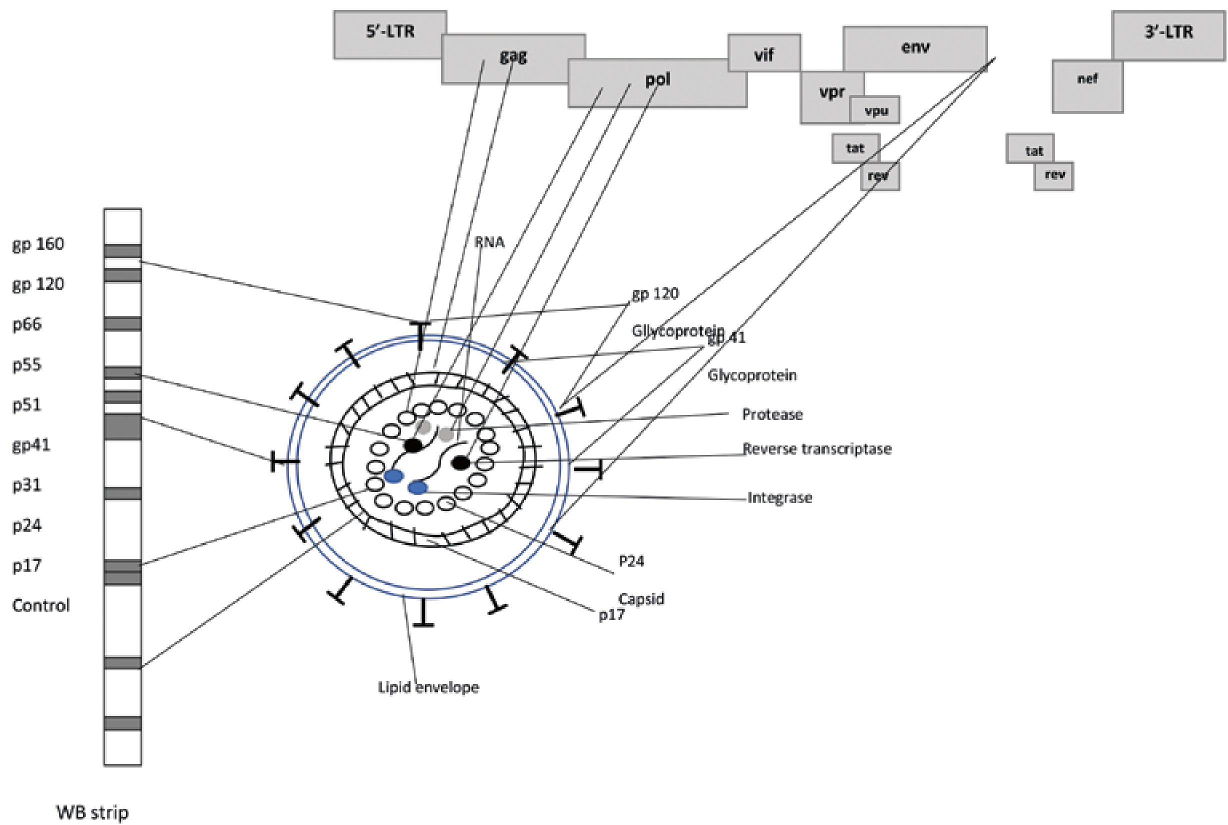

Figure 1. Organization of the HIV virion and the construction of the Western blot test (based on [40])

At the beginning of 1985, the FDA registered the first enzyme-linked immunosorbent assay (ELISA) for detection of anti-HTLV III/LAV antibodies [37]. In the same year, recommendations were issued for donors of blood and blood components to be screened for anti-HTLV III. According to these recommendations, all donations positive in screening tests were to be subjected to utilization and the donors notified of the positive test results. A test result was considered positive if repetitively reactive; each initially positive (reactive) test had to be repeated with some other available test, usually immunofluorescent or immunoprecipitation assays. In the years that followed, confirmatory testing was performed with Western blot (WB) reference test — a HIV antibody test. The first WB test was registered by the FDA on April 29, 1987 [38, 39].

Structure, genome organization, taxonomy

HIV virus belongs to the family *Retrovirdae*, genus *Lentiviridae*. So far, two types of the virus have been separated: HIV-1 and HIV-2 [40]. The virion is spherical in shape with a diameter of about 100 nm, consisting of a cylinder-shaped core and a lipid envelope (Fig. 1). Each virion contains 72 glycoprotein complexes that are integrated into the lipid membrane, and each complex consists of trimeric outer glycoprotein gp120 and trans--membrane splicing protein gp41. The bond between gp120 and gp41 is loose, and easily broken. The gp120 glycoprotein can be detected in both serum and lymphoid tissue of HIV-infected patients. Underneath the capsid is a layer of protein (p17). In the capsid, made up of the p24 protein, there is genetic material that takes the form of two single copies of ssRNA(+). In the virion, there are p6 and p7 proteins, two transport RNA (tRNA) molecules that are primers for cDNA synthesis, viral enzymes — reverse transcriptase (a heterodimer consisting of p66 protein having a domain with polymerase activity and RNAase H and p51 protein with a stabilizing effect), p10 protease and p32 integrase (Fig. 1). The HIV-1 genome includes nine genes. Three of them — gag, pol, env encode information needed for the production of structural and enzymatic proteins, while the others, i.e. tat, rev, nef, vif, vpr, vpu carry information on the construction

of regulatory proteins, which control HIV-1 ability to infect cells, produce progeny virions and disease progress. Long Terminal Repeat (LTR) sequences are located at both ends of the genome. These are regions responsible for regulation of viral gene expression [40, 41]. The organization of the viral structure is important for understanding the design and effectiveness of tests used for identification of infected donors. WB tests are based mainly on structural proteins of HIV (Fig. 1). As mentioned earlier, WB assays confirm the specificity of antibodies detected in blood donors in diagnostic and screening tests. In Polish blood transfusion service, the WB HIV results are interpreted in accordance with the American Red Cross guidelines, where a positive result is one in which antibodies to at least one viral protein are found in each of the three protein categories — envelope-forming, core and enzyme proteins. Now that HIV tests must be certified (CE IVD and number of notified body) [42, 43] the results are interpreted according to the manufacturers' leaflets. A negative result means that no antibody to any of the proteins was detected. An indeterminate result does not meet the criteria of either a positive or negative result and is most often due to non-specific reactions or indicates an early stage of infection [44–46]. Figure 1 presents the organization of the HIV viron and the construction of the Western blot test.

Evolution of the methodology of diagnostic and screening tests

The basic principle of enzyme immunoassays (EIA) or enzyme-linked immunosorbent assays-(ELISA) is binding of serum antibodies by antigens covalently bound to a solid phase surface, such as a microtiter well. After washing away the unbound antibodies the antigen-antibody conjugate is incubated with the substrate. In the presence of antibodies, a colored reaction pattern is produced which is monitored by spectrophotometric measures. The basic mechanisms of the I–III generation EIA assays are presented on the diagram in Figure 2. In the IV and V generation assays, not only solid phase antigens are coated but also specific anti-p24 antibodies. The sample tested for the presence of specific antibodies directed against the virus is added to the antigen-coated well (solid phase). If the sample contains antibodies directed against this antigen, an immune complex is formed and the antibody is permanently bound to the substrate. The next step is the washing out the non-specific antibodies from the tested serum/plasma. When the well is washed out, the so-called conjugate — an

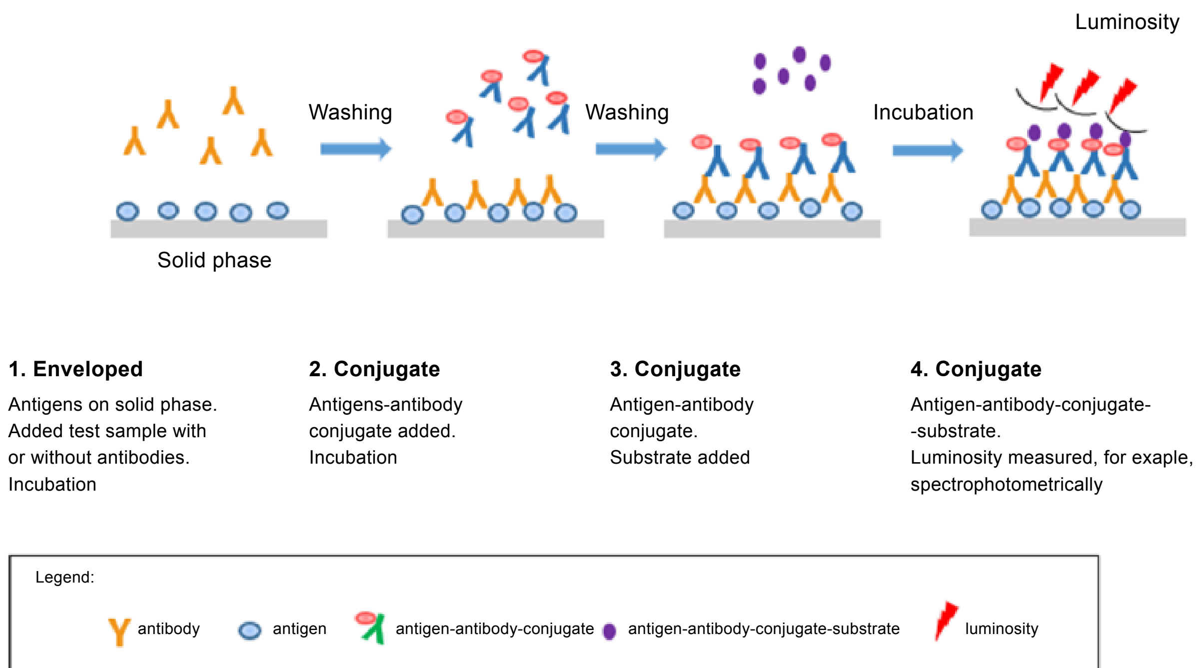

Figure 2. Scheme of the basic principles of the I–III generation ELISA/EIA test (based on [48], the term conjugate was used to describe an enzyme-antibody complex)

antibody (directed against human antibodies) linked to the enzyme by a covalent binding. Incubation and another rinsing follows and then a substrate proper for the enzyme is added to the well. The product of the enzymatic reaction (usually coloured) is assessed by appropriate methods (most often spectrophotometric measures) although in EIA assays chemiluminometry methods are also frequently used. The design of many commercial EIA assays tests is more complicated; assays of the latest generation enable detection of both HIV antibodies and antigens.

It is worth stressing that the light signal usually correlates with the amount of antibodies in the tested material. The specificity of antigen-antibody binding renders the results obtained with EIA tests highly reliable. Sporadically however, non-specific results are obtained as result of a cross-reaction when the antibody binds to another antigen [47, 48]. It is therefore important to have each ELISA reactive result verified with a confirmation test [47, 49].

The I generation assays used lysate from infected cells as the antigen source, and visualization was based on animal monoclonal antibodies which detect human IgG class antibodies [49, 50]. Abbott Laboratories was the first company which received FDA approval of the first licensed antibody test to identify HIV in blood [51]. In the years that followed, the ELISA assay was further developed. The II generation assays used glycoproteins or recombinant antigens instead of whole proteins. The specificity and sensitivity of the tests were thus increased [50]. III generation tests used a different EIA design. Recombinant HIV antigens placed in the solid phase were used to "capture" serum anti-HIV antibodies of both IgG and IgM class. Anti-HIV antibodies from patient's serum bind to recombinant or synthetic HIV antigens (the so-called antigen sandwich). The antibodies or antigen are covalently labeled with the enzyme or biotin/streptavidin to form the so-called conjugate (Fig. 2). The design further enhanced test specificity and enabled detection of IgM isotype s in addition to IgG class antibodies. The new tests allowed to detect antibodies about 21 days of infection which shortened the serological window period.

IV generation tests (combo), are able to detect both p24 antigen (HIV-1) and antibodies directed to HIV-1 and HIV-2. Antigen p24 is a distinctive HIV protein that makes up most of the HIV viral core or capsid. High levels of p24 are present in the blood serum of newly infected individuals during the short period between infection and seroconversion making p24 antigen assays useful in diagnosing primary HIV infection at a time when antibody levels are low [52]. The IV generation assay was designed/developed in 1989 by Abbott. The test contributed to shortening serological window period by 7 days (on average) as compared to III generation assays (up to 14 days) [50, 53–56]. In 2015, V-generation tests were implemented for HIV diagnosis which simultaneously detected both IgG and IgM class antibodies and the p24 antigen. The main difference as compared to IV-generation tests was the additional option of acquiring separate results for antibodies and the p24 antigen [50].

Western blot (WB) assays are used to verify tests reactive in EIA. WB is an enzyme immunoassay and the result presents in the form of bands which correspond to individual proteins (Fig. 1). The results can be read visually (no device necessary) or with the help of automated reader. WB tests determine the protein to which the antibodies were developed. The tests are of higher specificity than ELISA [57–59].

The algorithm for screening tests and verification tests in blood donors are outlined in the European guidelines [60] while for persons other than blood donors, the algorithm for HIV testing is presented in national guidelines [61–67]. In blood transfusion practice an initially reactive sample is retested in duplicate and if the result is repeat reactive a confirmatory test is performed using serological techniques and NAT (nucleic acid testing) [3–8].

Research on detection of the virus responsible for AIDS using molecular biology techniques was introduced in the United States in the second half of the 1980s [68]. Once the information on genome sequence of the virus became available it was possible to use it for blood donor screening. While analyzing the rationale for using such techniques it was emphasized however that, attention was focused on the fact, that although the HIV infection could be detected even before p24 antigen and anti-HIV antibodies appeared, the efficacy was low due to the low frequency of the expected positive results. Mathematical models demonstrated that in the U.S. HIV RNA donor testing would prevent 16 cases of viral transmission per year while the cost of such screening would amount to $96 million [69]. Technical difficulties related to NAT testing were also emphasized. In the years that followed it was demonstrated that the implementation of screening by NAT is technically feasible and absolutely justified because it contributes to the strengthening of transfusion safety [70]. Further

HIV-1

Figure 3. Timetable of the introduction of NAT blood donation testing up to 2010 — by virus and country (based on [72])

advancement of molecular biology technology as well as development of multiplex assays for simultaneous detection of genetic material of several viruses, rendered mass screening in blood transfusion more accessible. At the same time, many countries reported numerous cases of transfusion transmitted HIV infections [71]. Several countries implemented NAT testing for blood donors. According to current national and international guidelines, NAT techniques should detect at least 10,000 IU/mL of HIV RNA per single donation [3–8]. Figure 3 presents the implementation of NAT for HIV by country.

Further evolution was stimulated (among others) by data from Germany; in the period 2007–2010, false-negative NAT results which detected a single region of HIV-1 RNA were reported in 6 blood donors. To improve the diagnostic sensitivity of HIV-1 RNA , especially of the polymorphic forms, tests were introduced that simultaneously detected at least two conserved regions (dual-target assay) of the viral genome. German studies of 2010–2014 demonstrated higher clinical sensitivity of HIV detection with dual-target assays (amplification of two regions of the viral genome) as compared to those that analyze a single region [72, 73].

The principles and diagnostic algorithm of HIV detection in the general population, differ slightly from those in blood donor screening. According to the recommendations of the Polish AIDS Scientific Society (PTN AIDS), the former type of testing should be based on serological tests. Third- and fourth-generation tests as well as the so-called rapid tests are recommended [61–65]. As of 2017, PTN AIDS recommends using IV generation tests for donor screening. In exceptional cases, HIV-1 RNA testing is allowed [66, 67]. A negative screening test 12 weeks after exposure rules out HIV transmission.

When a reactive result is obtained, the test should be repeated in duplicate; in the case of repeat reactive result, a confirmation test is required. The patient should be informed as soon as possible and informed on prevention of HIV transmission as well as referred to an outpatient clinic or hospital [74].

Evolution of HIV screening methodology in Poland

Anti-HIV testing of blood donors in Poland began in 1986 — about 5% of donations were then tested. According to the recommendation of the Minister of Health and Social Welfare, the detection of anti-HIV antibodies were to trigger widespread screening in blood transfusion service. Anti-HIV-antibodies were first detected in September 1986 when blood was collected from a HIV-infected donor at the Blood Transfusion Center in Opole (IHTM data). Since November 1987, mass blood donor screening was performed with II generation tests, which detected only anti-HIV-1 antibodies 42 days of exposure (on average). At the same time, new methods were evaluated and procedures developed for correct and reliable interpretation of results [75]. The first recommendations for blood transfusion service regarding HIV/AIDS prophylaxis and diagnosis were issued in Poland in 1989 with the aim of standardizing diagnostic procedures for HIV infection [76]. In the years that followed donor screening was performed with III generation tests. In 2000, IV generation EIA tests were implemented which detected antibodies and antigens. Another step towards better safety of blood and blood components was the implementation in the first quarter of 2007 of IV generation chemiluminescent tests using a fully automated apparatus. Full automation reduced the risk of human error during testing [77].

In Poland, HIV genetic material was first tested in donors with indeterminate results in Western blot and in individuals exposed to HIV-infected material. Viral DNA integrated with host DNA was tested and no HIV genetic material was detected [78]. NAT screening in blood donors for HIV RNA

which detected infection before serological markers appeared was implemented in Poland in the second half of 2003. At first, only some BTCs performed this screening using Procleix test (mandatory at the time for HCV RNA) which could also be used for HIV-1 RNA detection. Moreover, since 2003, HIV RNA testing became an additional procedure apart from Western blot, used for confirmation of repeat reactive results in serological tests [79]. In 2005, HIV RNA testing became mandatory for every donation [77, 79].

Sample collection for HIV screening

Blood samples for pathogen testing are collected during or after the donation from the same venipuncture and into disposable tubes (vacuum system). Samples from donor candidates are collected during the donor qualification procedure.

Only negative test results qualify blood or blood components for clinical use [3–8].

Methods

Serological tests, were always performed in single donations; NAT assays in single donations or minipools of equal volume plasma from "n" donor samples (n = 24 or 8 or 6). The tests and equipment/apparatus were CE IVD marked/certified. Screening tests were performed according to IHTM recommendations as presented in the subsequent versions of the "*Medical Principles for Collection of blood, Separation and issue of Blood Components for Organizational Units of the Public Blood Transfusion Service"* [3–5] and subsequent legal acts [6–8]. International regulations do not specify the principles of test design; according to the Directive, tests should detect the most common subtypes, mutants, recombinant forms, as well as newly emerging forms of the virus [42].

Serological tests

According to national and international recommendations [3–9], the analytical and clinical sensitivity and specificity of serological tests was close to 100% and > 99.5% respectively. From 2005 to 2022, tests from various manufacturers were used in BTCs for blood donor screening. Figure 4 presents the serological methods used for donor screening at individual BTCs in Poland in 2005–2022.

The algorithm for HIV immunochemical testing used throughout the period under analysis is shown in Figure 5. It was described in detail in the successive versions of the recommendations in effect in blood transfusion service (the *Medical Principles* [3–5] and the later *Good Practices* [6–8].

According to current Polish regulations, an initially reactive result (IR) in the screening test had to be repeated in duplicate from the same sample on the following day. If repeat reactive result (RR) is obtained (at least two positive results in three tests), the donor' blood sample was sent to IHTM for confirmatory assay.

NAT screening for HIV RNA

Since its implementation in the Polish blood transfusion service, NAT testing has been performed with two alternative methods: PCR (Polymerase Chain Reaction) and TMA (Transcription Mediated Amplification). The essence of PCR is the *in vitro* multiplication (amplification) of a selected genome fragment using thermostable DNA polymerase (Fig. 6). HIV is an RNA virus, therefore the first step must be reverse transcription. For this purpose a reverse transcriptase or DNA polymerase with both polymerase and reverse transcriptase properties are used. PCR is a cycling process — each cycle consists of three steps that differ as regards temperature. Repetition of the cycle doubles the number of the amplified DNA fragments. In reality, the reaction is slightly less effective mainly due to lower activity of the polymerase enzyme used in PCR. Specificity of the process is determined by proper selection/design of primer sequences, probes and temperature of reaction. The outcome is a copy of the tested DNA fragment of precise length and sequence [46]. A variation of the PCR method is RT-PCR (real time Polymerase Chain Reaction), which detects the amplification in actual/real time. The transcription mediated amplification (TMA) reaction uses transcription as a method to amplify nucleic acids. The composition of the reaction mixture is similar to that in PCR but here two enzymes are used: murine reverse transcriptase (RT) and bacteriophage T7 RNA polymerase. A schematic of the TMA reaction is shown in Figure 7 [46]. The efficiency of TMA and PCR reactions is very high – within an hour the number of nucleic acid molecules is multiplied up to 10^9 times. Tests performed in blood donations are fully automated which markedly reduces the risk of false results due to human error.

Table 1 presents the characteristics of the tests used for HIV RNA screening. HIV RNA testing was performed at the Molecular Biology Laboratories (PBMs) of the Blood Transfusion Centers. The Centers that had no PBM outsour-

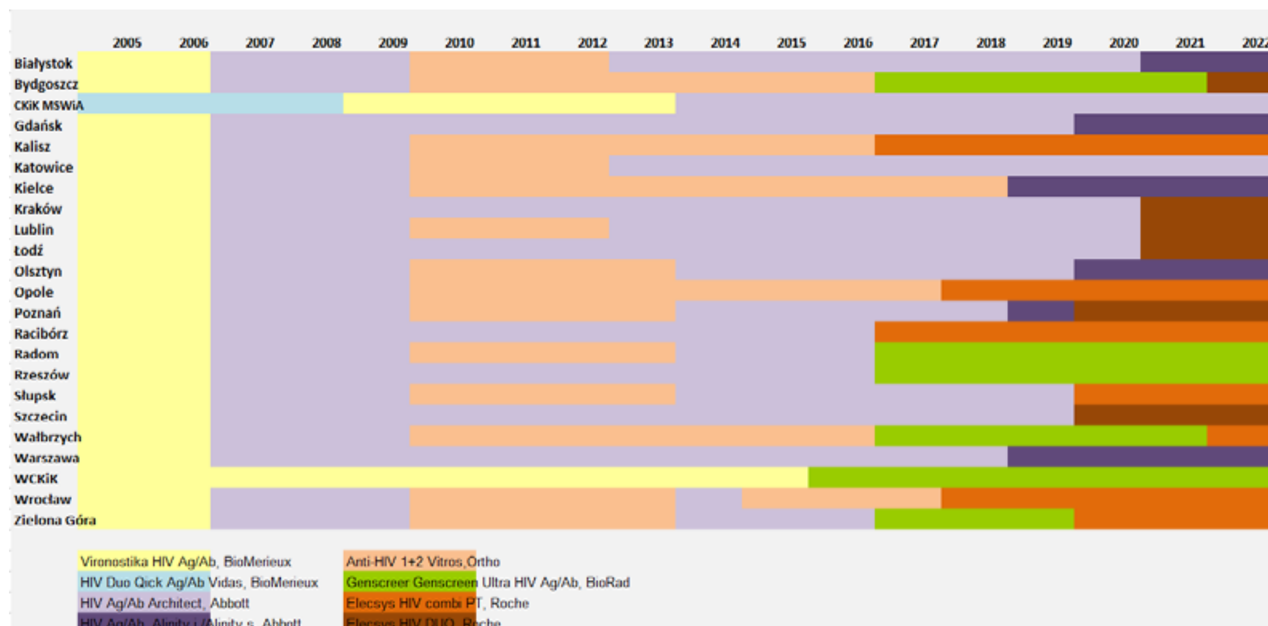

Figure 4. Serological screening methods used in Polish blood transfusion service (2005–2022)

ELISA — enzyme-linked immunosorbent assay; ELFA — enzyme-fluorescent assay; CIMA — chemiluminescent assay; ECI — enzyme-linked immunosorbent assay; ECLIA — electrochemiluminescent assay; CKiK MSWiA — Blood Transfusion Center of the Ministry of Internal Affairs and Administration, supervised by the Ministry of Internal Affairs and Administration

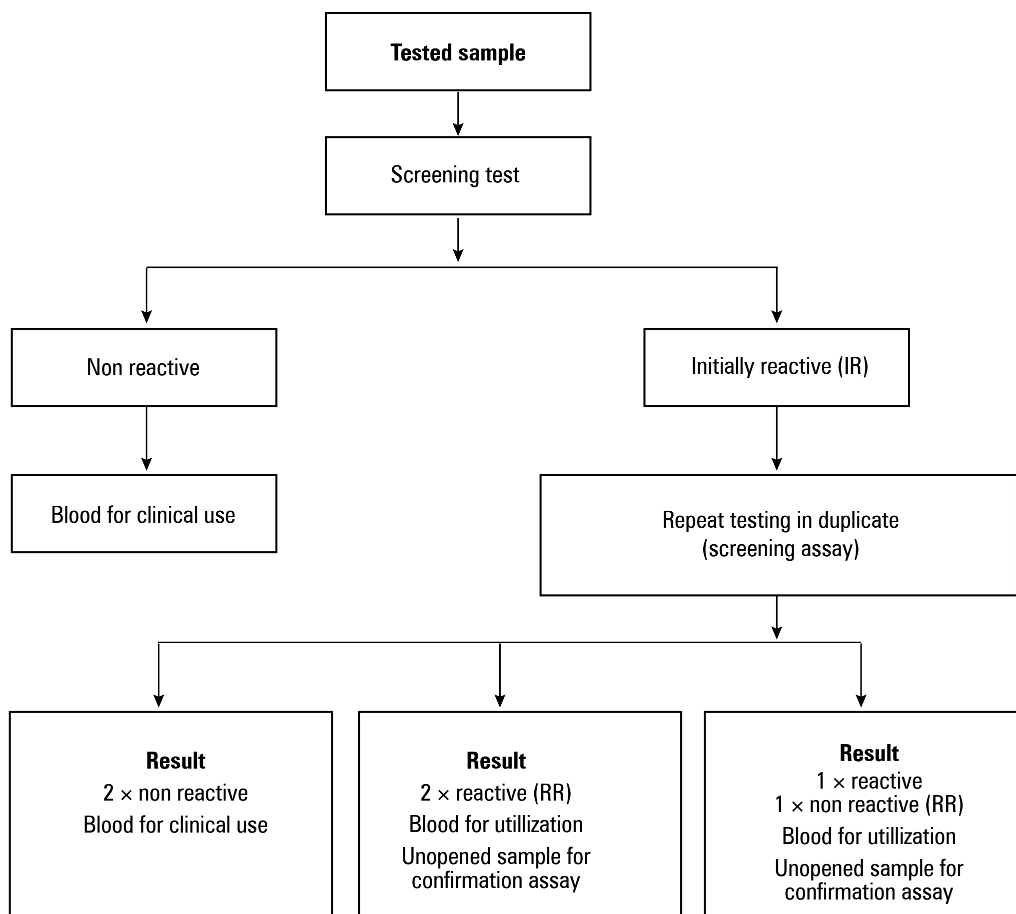

Figure 5. Algorithm of screening tests (serological tests) performed in Blood Transfusion Centers (BTCs)

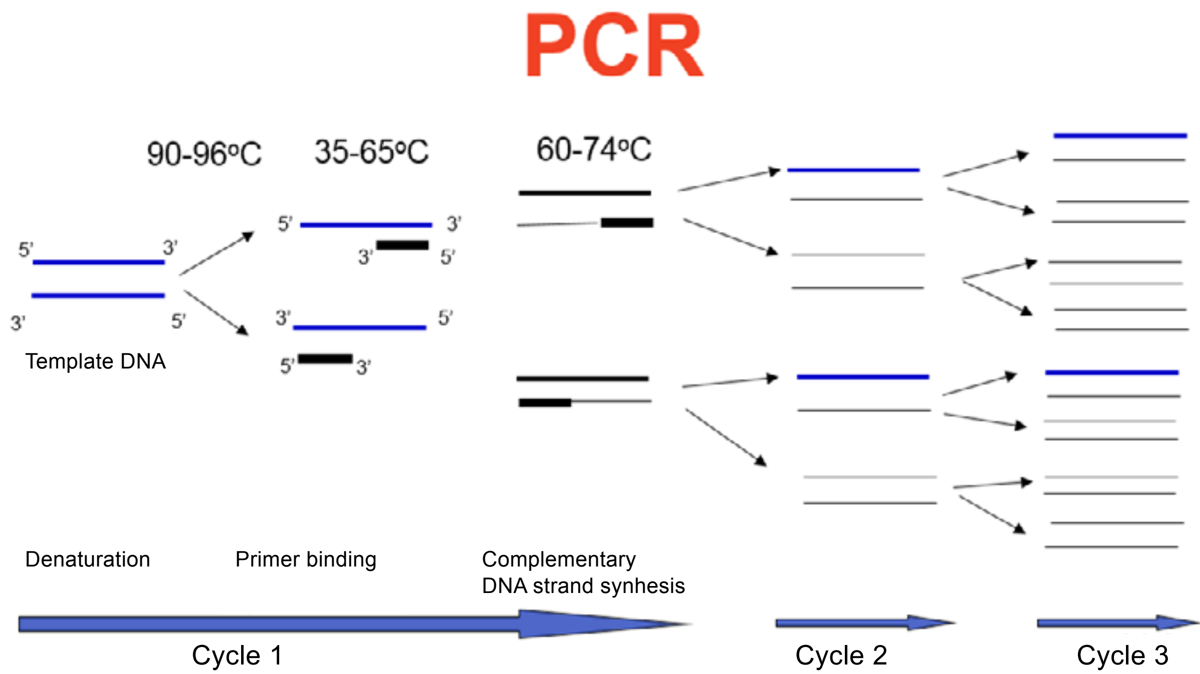

Figure 6. PCR reaction scheme, (based on [46])

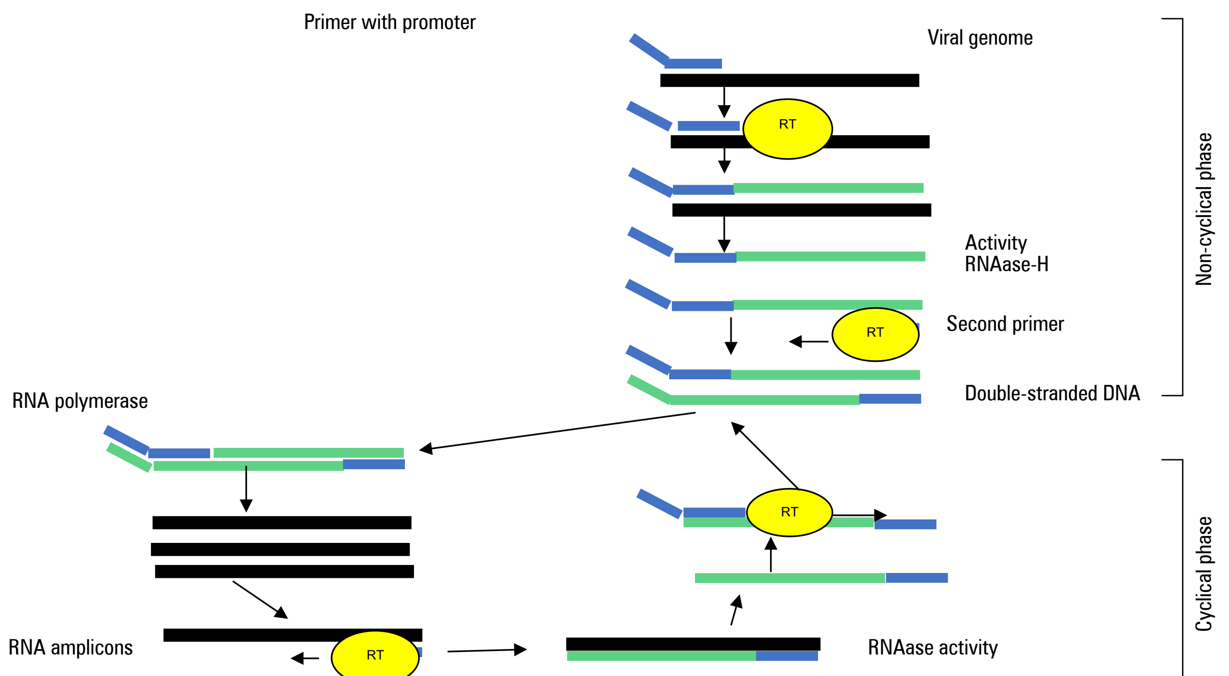

Figure 7. TMA reaction scheme, (based on [46])

Table 1. Characteristics of RNA HIV screening in BTC and confirmatory test in BTCs in the period 2005–2022 (sensitivity data based on IFU)

Test	Format	Sensitivity for donation IU/ml (95%LOD)	Differential tests	Used
TMA				
Procleix Ultrio		45	Yes	2005–2009
Procleix Ultrio Plus	IDT	27.6	Yes	2010–2013
Procleix Ultrio Elite		18	Yes	2014–2022
TMA				
Procleix Ultrio Plus	MP 8	221	Yes	2012–2014
PCR				
Ampliscreen HIV	MP 24	1469	No	2005–2007
R-T PCR				
Taqscreen MPX		294	Yes	2007–2012
Taqscreen MPX v.2.0	MP 6	302	Yes	2013–2018
MPX		154,2	Yes	2019–2022
TMA				
Procleix Ultrio Elite	MP 4	72	Yes	2017–2022

IDT — study in a single donation; the number next to MP indicates the number of donations in mini pool e.g. MP 8 — study in a mini pool of 8 donations

ced the service to a BTC that had. Information on NAT screening assays used at BTCs is shown in Figure 8. Two strategies were used for screening with molecular biology techniques:

a) in single donations (ID) or
b) in mini-pools (MP) of plasma from "n" donations.

The methods used were:

— TMA in:
  - single donations (ID) or
  - minipools of 8 donations (in 1 BTC in 2012–2014) or
  - minipools of 4 donations (4 BTCs since 2022);

— PCR in minipools of:
  - 24 donations (2005–2006) or
  - 6 donations (since 2007).

All screening tests except *ampliscreen HIV* were multiplex which allowed simultaneous detection of HIV and HCV RNA and HBV DNA. A reactive result in a multiplex test required additional differential testing to identify the virus responsible for the reactive result. An exception here was the Taqscreen MPX v. 2 test in which a reactive result was immediately obtained with an indication of the virus responsible. Once a reactive result was obtained for a TMA screened pool, single donations were tested using differential tests for each virus (HIV-1/2, HCV, HBV). If a reactive result was obtained in single donations, testing was performed in duplicate with differential tests. A nonreactive result did not defer the donor but blood components from such a donation could not be used for clinical purposes. The flowchart for each test is shown in Figure 9.

When an initially reactive result was obtained in PCR (in 24 or 6 donation pools), further tests were performed in the BTCs. For 24 donation pools, the next step was the testing of 4 subpools of 6 donations each. Once a reactive result was obtained in a 6 donation pool (subpool), testing was continued in the single donations of this subpool. Donations from the negative subpool were qualified for clinical use. The testing algorithm is shown in Figure 10.

Evolution of NAT screening strategies in BTCs

The analytical sensitivity of NAT tests used for HIV RNA screening in the Polish blood transfusion service has evolved as more assays were introduced and the assay format changed (MP24, 6, 4, IDT). In 2005–2006, the sensitivity of the MP24 test was estimated at 1469 IU/mL [95% LOD]. Following implementation in 2007 of the Cobas Taqscreen assay in MP6 the analytical sensitivity increased several-fold to approximately 300 IU/ml. The analytical sensitivity of TMA in single donations was very high from the very beginning for: Procleix Ultrio (2005–2009 — 45 IU/ml [estimated 95% LOD], for Procleix Ultrio Plus (2010–2013) 27.6 IU/ml and the highest for the Procleix Ultrio Elite test (used since 2014 until now) — about 18 IU/ml. Data presented in Figure 11.

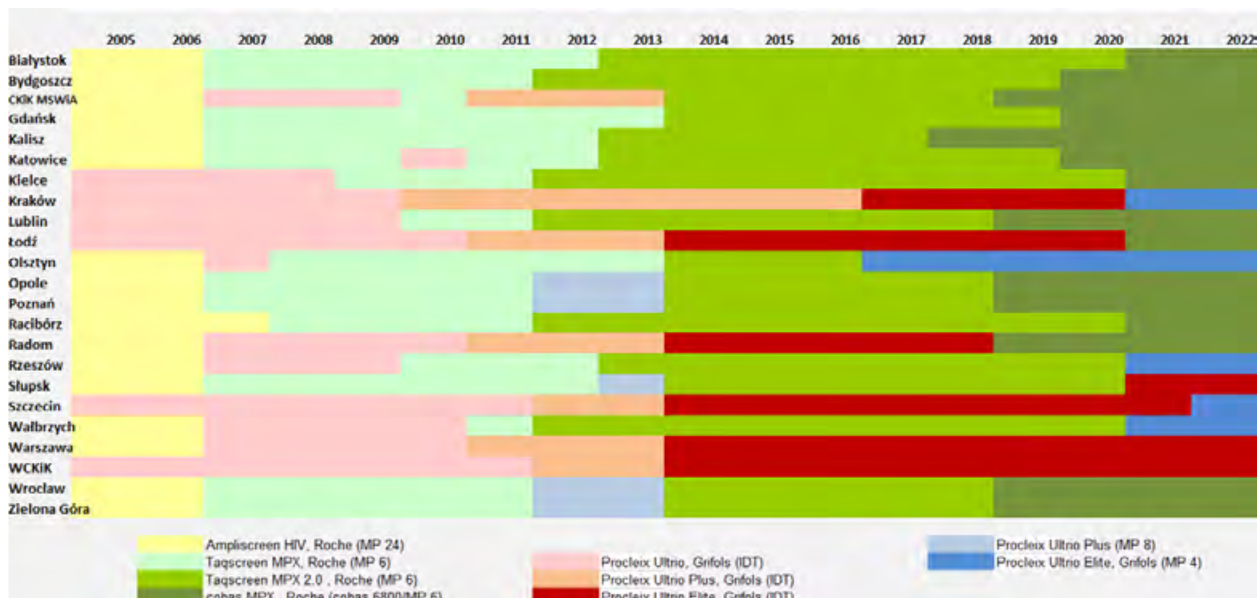

Figure 8. Molecular biology screening methods used in Polish blood transfusion service in the period 2005–2022; CKiK MSWiA — Blood Transfusion Center of the Ministry of Internal Affairs and Administration, supervised by the Ministry of Internal Affairs and Administration

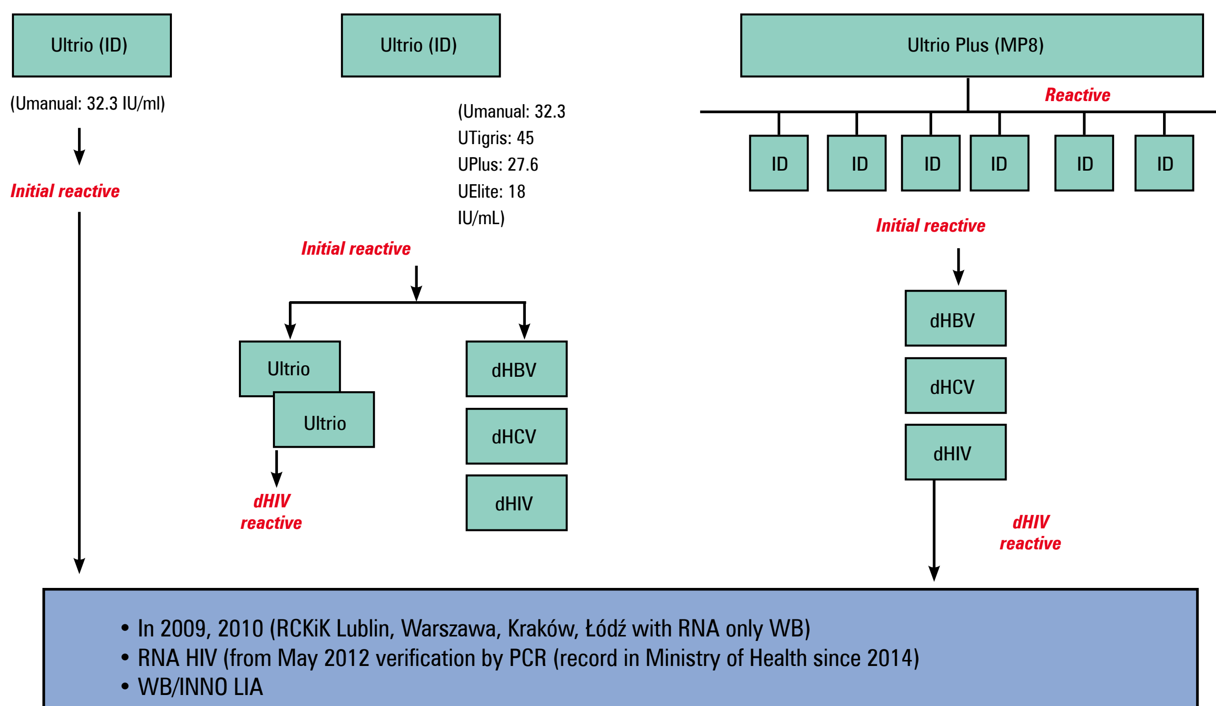

Figure 9. Procedure for reactive result in HIV RNA screening — TMA (sensitivity data based on IFU)
ID — individual donation; dHBV — dyscrimination HBV; dHCV — dyscrimination HCV; dHIV — dyscrimination HIV;
RNA — ribonucleic acid; WB — western blot test; MZ — Ministry of Health; WB/INNO LIa — western blot test;
PCR — polymerase chain reaction

Quality control of screening tests performed in BTCs (2005–2022)

The BTC laboratories which performed screening for transfusion transmitted pathogens followed the procedures listed below to ensure reliability of results:

- screening was preceded by evaluation of equipment and tests as well as validation of processes;
- process validation was conducted in each BTC immediately prior to screening with sample panels prepared by the Department of Virology

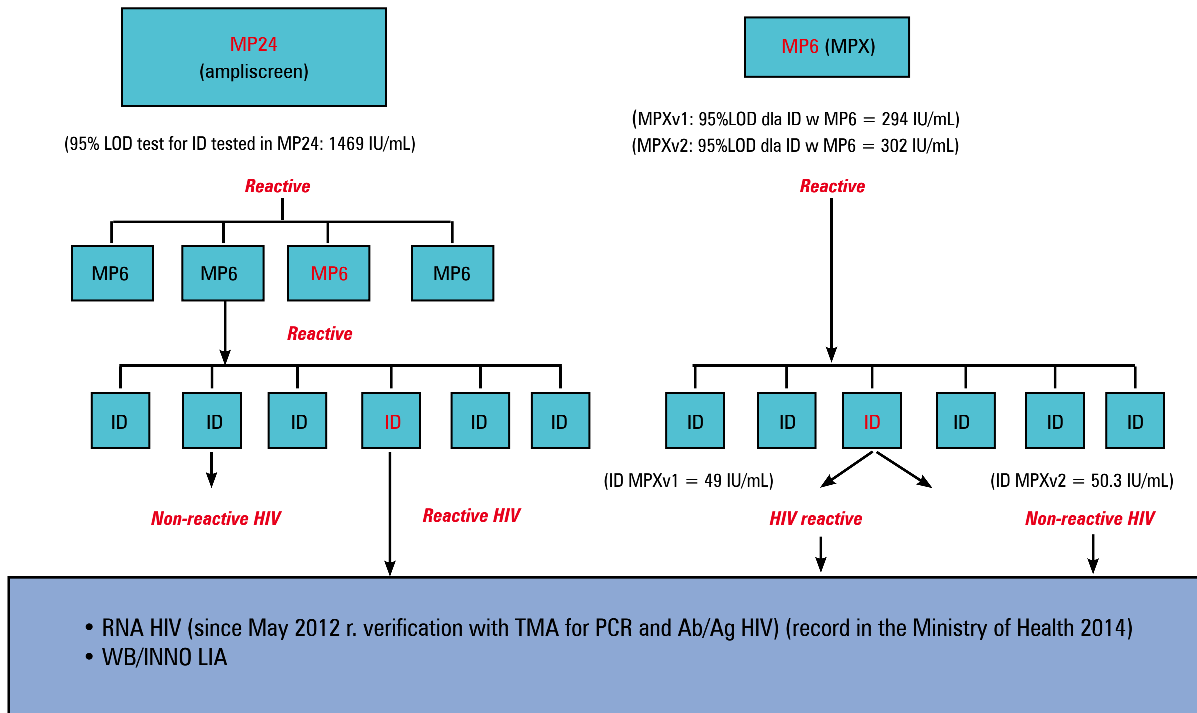

Figure 10. Procedure in Blood Transfusion Centers for reactive result in PCR (sensitivity data based on IFU) MP24 — minipool 24 donations; MP6 — minipool 6 donations; ID — individual donation; RNA — ribonocleic acid; HIV — human immunodeficiency virus; TMA — transcription mediated amplification; PCR — Polymerase Chain Reaction Ab/Ag HIV — antibody/antigen HIV; MZ — Ministry of Health; WB/INNO LIA — western blot test

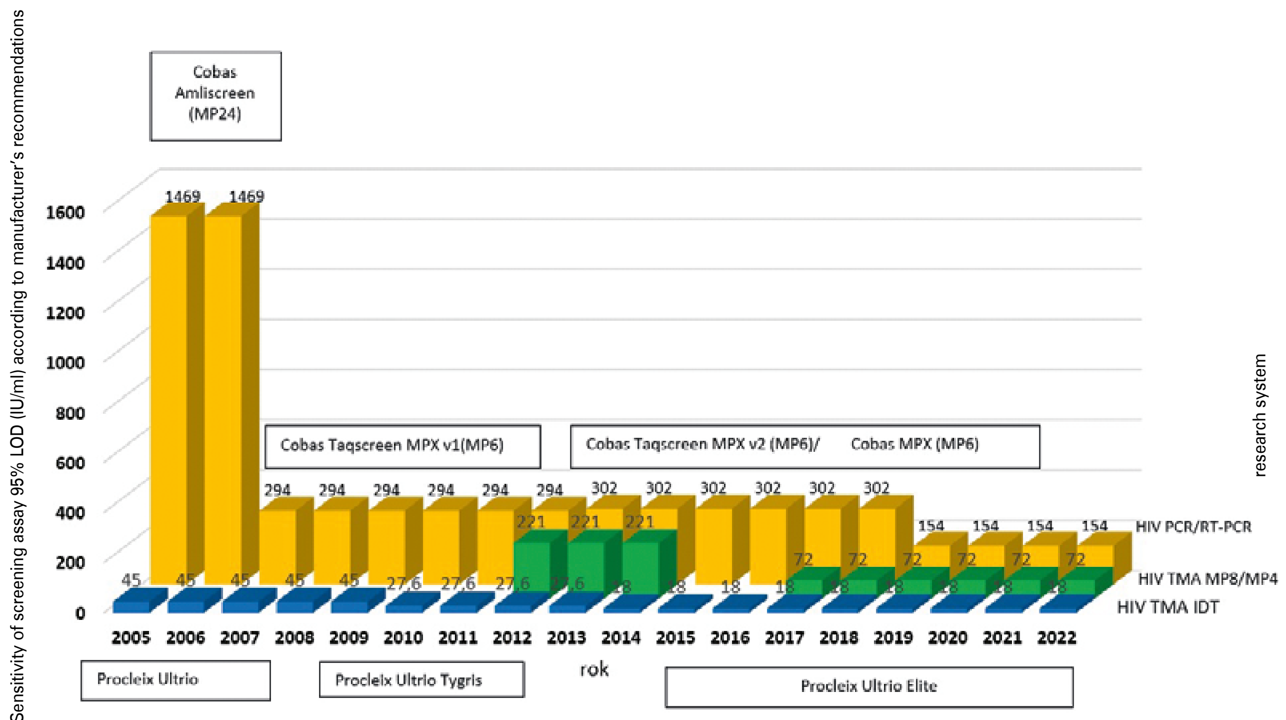

Figure 11. Analytical sensitivity of NAT tests for HIV RNA in Polish blood transfusion service in the years 2005–2022 (sensitivity data based on IFU)

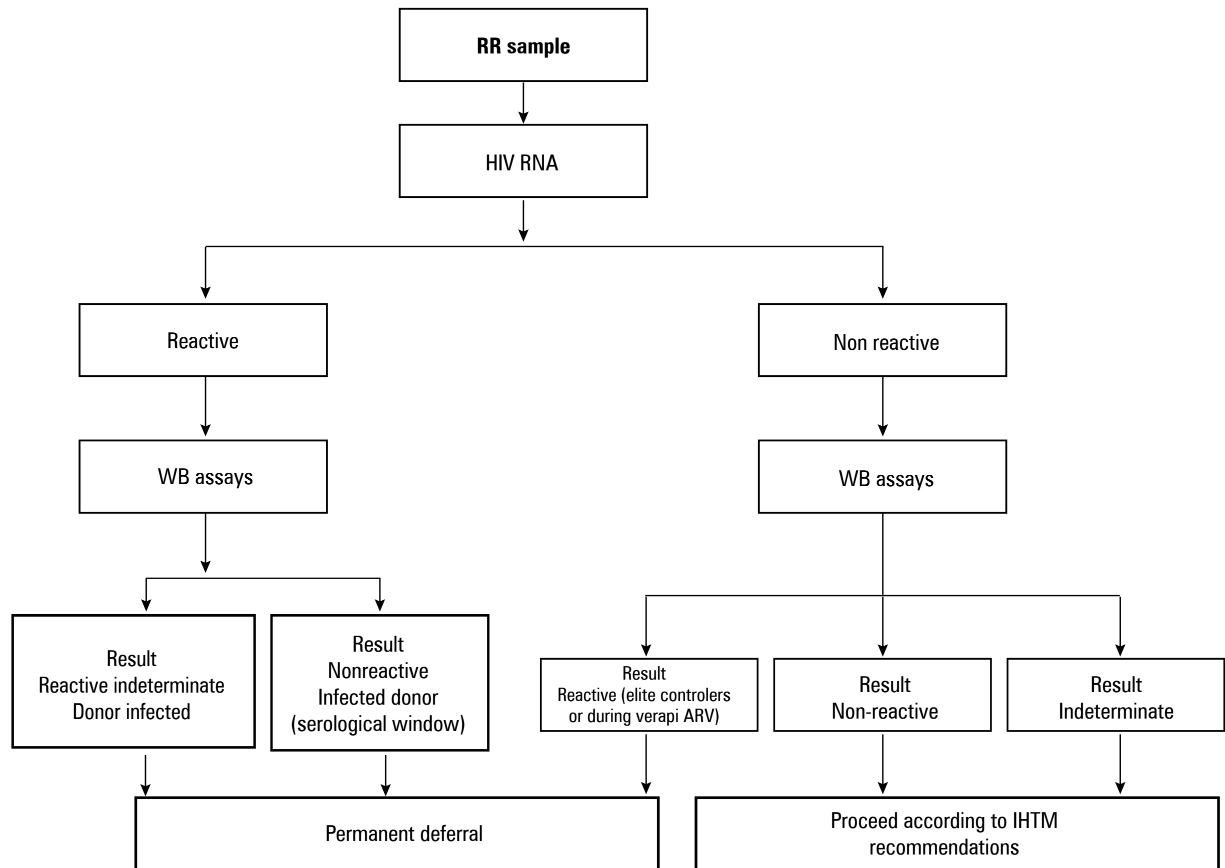

Figure 12. Algorithm of the verification procedure following repeat reactive HIV-1/2 result in screening tests

(IHTM); participation in such quality control program was repeated every 12 months;
- screening tests were performed strictly according to manufacturer's requirements;
- all laboratories participated in the external quality control program as well as in the EDCNet program of daily external laboratory quality control;
- all laboratories were audited regularly every two years.

Confirmatory tests

Samples which were repeat reactive in serological screening or/and NAT were sent to the Department of Virology (IHTM for verification/ /confirmatory testing.

Confirmatory tests serve to confirm or exclude HIV infection — samples which were repeat reactive in serological screening or/and NAT were tested in Western blot (WB) and HIV genetic material was additionally tested (always in single donations). The flowchart for the verification process is shown in Figure 12. In May 2012, the algorithm for confirmatory NAT testing was supplemented by mandatory testing of HIV RNA with a method other than the one used for screening. If screening was performed with PCR, the TMA method was used for confirmatory testing at IHTM; if screening was performed with TMA, the verification test was performed with PCR. This recommendation served to detect polymorphic forms which remained undetected by NAT. The intention was to eliminate false negative results (the so-called escape mutants).

In cases of unconfirmed infection, the first, 6-month temporary deferral was imposed on the donor. Subsequent indeterminate results of confirmatory tests resulted in extension of deferral by another 6 months to more than two years. The donor could be restored to donating blood when the results of both screening and confirmatory tests were negative. If non-specific results persisted

Table 2. WB tests used at the IHTM for confirmatory testing of HIV infection in the years 2005–2022

Years	Test (manufacturer, country)
2005–2006	HIV BLOT 2.2 (Genelabs® Diagnostics, Singapore)
2007–2008	HIV-1 BLOT 1.3 (Genelabs® Diagnostics, Singapore)
2009	HIV BLOT 2.2 (Genelabs® Diagnostics, Singapore)
2010	INNO-LIA™ HIV I/II Score (Innogenetics, Belgium)
2011–2012	HIV BLOT 2.2 (MP Diagnostics, Singapore)
2013	HIV BLOT 2.2 (MP Diagnostics, Singapore) INNO-LIA™ HIV I/II Score (Innogenetics, Belgium)
2014	INNO-LIA™ HIV I/IIScore (Innogenetics, Belgium)
2015–2022	*recom*Line HIV-1& HIV-2 IgG (Mikrogen, Germany
From 10.2022	INNO-LIA™ HIV I/IIScore (Fujirebio, Belgium)

Table 3. NAT tests used in verification studies at the IHTM in the years 2005–2022 (sensitivity data based on IFU)

Years	Test (manufacturer/m country)	Sensitivity to donation IU/ml (95%LOD)	Number of amplified regions
2005–2006	Procleix Ultrio (Gen-probe, USA)	32.3 IU/ml	No data*
2007–2009	Procleix Ultrio (Gen-probe, USA)	32.3 IU/ml	No data*
	Cobas Ampliscreen HIV-1 v 1.5 (Roche, USA)	61.2 IU/ml	No data*
2010	Procleix Ultrio (Gen-probe, USA)	32.3 IU/ml	No data*
	Procleix Ultrio Plus (Gen-probe, USA)	28.6 IU/ml	No data*
	Cobas Ampliscreen HIV-1 v 1.5 (Roche, USA)	61.2 IU/ml	No data*
2011–2013	Procleix Ultrio Plus (Gen-probe, USA)	28.6 IU/ml	No data*
	Cobas Ampliscreen HIV-1 v 1.5 (Roche, USA)	61.2 IU/ml	No data*
2014	Procleix Ultrio Elite (Gen-probe, USA)	18 IU/ml	2
	Confirmatory PCR Kit HIV-1 v 1.1 (GFE Blut, Germany)	89.5 IU/ml	3
2015–2021	Procleix Ultrio Elite (Gen-probe, USA)	18 IU/ml	2
	Confirmatory PCR Kit HIV-1 v 1.2 (GFE Blut, Germany)	13.7 IU/ml	3
From 11.2021	Procleix Ultrio Elite (Gen-probe, USA)	18 IU/ml	2
	artus® HI Virus — 1 RG RT-PCR KIT (Qiagen, Germany)	66.9 IU/ml	1

*No information in test flyer/leaflet

for longer periods of time, long-term temporary deferral (2–5 years) was imposed on the donor. Permanent deferral was imposed on the donor only after HIV infection was confirmed.

During the period covered by the current analysis, WB tests from various manufacturers were used for serological confirmation assays (Table 2). Tests used for HIV-1/2 RNA detection in verification testing are presented in Table 3.

Residual risk of transfusion-transmitted HIV infection; how to reduce the risk

Despite screening tests, the risk of transfusion transmitted HIV infection still exists (residual risk). One way to reduce this risk is the deferral of donor candidates and donors who have reported behavior and circumstances associated with higher risk of HIV infection in the pre-donation

questionnaire or during medical interview. Implementation of serological screening tests and NAT has dramatically increased the safety of blood and blood components. Bruhn et al. conducted estimates of screening efficacy (assuming alternatively two values of infectious dose ID_{50} = 3.16 viremia and ID_{50} = 316 viremia) for the RBCs in donations from first-time and multiple donors for different screening scenarios worldwide [80].

Screening efficacy in donations from first--time donors increased from 98.73% for screening performed with anti-HIV antibody detecting tests to 99.78% for both ID-NAT and antibody tests; in donations from multiple donors the screening efficacy ranged from 86.7% for anti-HIV antibody tests to 97.68% for the more effective ID-NAT/Ab + testing strategy [80]. Lelie et al. estimated the diagnostic window period for the Ultrio Elite test on the Panther platform (single donation) at 4 days, and for the MPX test on the cobas 6800 platform (6 donation minipool) at 5.7 days. Currently, the estimated residual risk is < 1/1,000,000 donations and defined as the risk of transfusion transmitted infection despite the use of screening tests, all control measures and good practices for multiple donors in developed countries.

Current status and the future of blood donor testing for HIV

The current screening and verification pattern seems to be sufficient to ensure transfusion safety of blood and blood components. The analysis of residual risk demonstrates that further increase of the sensitivity of NAT testing will not contribute much to blood safety. The level of residual risk is acceptable and does not justify further financial investment to increase test sensitivity.

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