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HCV RNA and HIV RNA detection by Procleix HIV-1/ /HCV Assay in blood donors with various results of anti-HCV and anti-HIV EIA

Wykrywanie RNA HCV i RNA HIV metodą Procleix HIV-1/HCV u dawców krwi z różnymi wynikami immunoenzymatycznych badań przeglądowych anty-HCV i anty-HIV

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Summary

Background: *The aim of the study was to investigate the sensitivity of Procleix HIV1/HCV Assay for HIV RNA and HCV RNA detection, and to analyse the frequency of viral nucleic acid detection in blood donors with various EIA test results.*

Material and methods: *The 95% sensitivity of the Procleix test was 6.2 IU HCV RNA /ml and 44.5 IU HIV RNA /ml. HCV RNA was detected in 71/392 (18.1%) anti-HCV positive and HIV RNA in 21/557 (3.8%) EIA HIV-reactive donors. The frequency of HCV RNA detection correlated with signal/cut-off ratios (S/C ratio) of EIA. HCV RNA was found in 68/105 (64.8%) of the donors if it was >4, in 1/85 (1.2%) if it was between 2.00 and 3.99, and in 2 out of 202 donors (1%) if it was between 1.00 and 1.99. HIV RNA was detected in all 21 blood donors positive in EIA and Western Blot.*

Results and conclusions: *The study demonstrated that Procleix HIV1/HCV Assay is very sensitive and can be used in the reference laboratory to confirm active infection in donors with positive results. We observed a low frequency of active infection in Polish blood donors with repeated reactive results in HCV and HIV EIA. The high S/C ratio value (> 4) of EIA is a good predictor of HCV RNA detection, but HCV RNA can also be detected in single donors with low ratio values of the EIA test.*

Key words: Anti-HCV, anti-HIV, HIV RNA, HCV RNA, transcription-mediated amplification

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Streszczenie

Wstęp: *Celem pracy była ocena czułości wykrywania RNA HIV i RNA HCV metodą Procleix HIV1/HCV oraz analiza częstości wykrywania materiału genetycznego wirusów u dawców z różnymi wynikami testów przeglądowych anty-HCV i anty-HIV.*

Materiał i metody: *Dziewięćdziesięciopięcioprocentowa czułość testu Procleix wynosiła 6,2 IU RNA HCV /ml i 44,5 IU RNA HIV /ml. RNA HCV wykryto w 71/392 (18,1%) dodatnich próbkach anty-HCV, zaś RNA HIV w 21/557 (3,8%) próbkach reaktywnych w badaniu EIA HIV. Częstość wykrywania RNA HCV korelowała z wartością S/C uzyskaną w badaniach immunoenzymatycznych. RNA HCV wykryto u 68/105 (64,8%) dawców z S/C > 4, u 1/85 (1,2%) z S/C od 2,00 do 3,99 i u 2/202 dawców (1%) z S/C od 1,00 do 1,99. RNA HIV wykryto u wszystkich 21 dawców z dodatnimi wynikami zarówno w badaniu EIA, jak i w Western Blot.*

Wyniki i wnioski: *Procleix HIV1/HCV jest bardzo czułym testem i może być wykorzystywany w laboratorium referencyjnym do potwierdzania aktywnego zakażenia u dawców z dodatnim wynikiem immunoezymatycznych badań przeglądowych. Obserwowano małą częstość aktywnych zakażeń u dawców z powtarzalnie dodatnimi wynikami badań anty-HCV i anty-HIV. Wysoka wartość S/C (> 4) w EIA jest dobrym czynnikiem prognostycznym wykrycia RNA HCV, aczkolwiek RNA HCV może być sporadycznie wykrywane u dawców z niższą wartością S/C w EIA.*

Słowa kluczowe: Anty-HCV, anty-HIV, RNA HIV, RNA HCV, amplifikacja przez transkrypcję

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Introduction

Screening blood donations for anti-HCV and anti-HIV antibodies prevents transfusion-transmission of the hepatitis C virus (HCV) and human immunodeficiency virus (HIV). In Poland, in the first year of anti-HCV screening (1994), 1.4% of blood donors were found positive [1] and 0.14% were found positive in 2003 (unpublished data). However, not all anti-HCV positive donations were infectious [2]. According to Polish regulations, all anti-HCV and anti-HIV reactive blood donors are deferred from the donor file and directed to a general practitioner for further monitoring. Diagnostic confirmatory and supplementary procedure and subsequent infectious marker monitoring are performed in the Reference Laboratory at the Institute of Haematology and Transfusion Medicine in Warsaw [3]. All anti-HIV positive sera are tested by Western Blot to confirm the specificity of antibodies whereas anti-HCV positives in all regular donors are tested by Cobas Amplicor for HCV RNA to confirm the active infection. In most HCV RNA negative samples, a supplementary Western Blot test (RIBA) is also performed.

The aim of the study was to validate the Procleix HIV1/HCV Assay and to assess its usefulness for HIV RNA and HCV RNA detection in blood donors with positive results of EIA tests. We performed a comparative evaluation of HIV/HCV NAT with screening HCV and HIV EIA assays used in the Polish blood service.

Materials and methods

The analytical sensitivity of the Procleix HIV-1/ /HCV assay was determined by testing serial dilutions of WHO International Standards: HCV RNA (NIBSC 96/7901 — genotype 1) and HIV RNA (NIBSC 97/656) (Table 1). Estimations of the 95% and 50% detection limits of the assay were performed using Probit analysis.

In addition, the sensitivity of the Procleix assay against HCV genotypes 3a and 4c/d was analysed by testing 4 dilutions in 8 repeats of 3 samples anti-HCV neg/ HCV RNA pos for each genotype. The HCV genotypes in those samples were investigated by InnoLipa HCV (Innogenetics, Belgium) and the level of the virus by quantitative Amplicor HCV Monitor™ version 2.0 (Roche Diag, Switzerland) (Table 2).

The specificity of the Procleix Assay was estimated by simultaneously testing 291 anti-HCV/HIV negative, RNA HCV/HIV negative samples with

Table 1. HCV and HIV concentrations in WHO standard dilutions used to determine the analytical sensitivity of the test

Tabela 1. Koncentracja HCV i HBV w rozcieńczeniach standardu WHO użytych do oceny czułości analitycznej testu Procleix HIV1/HCV

HCV RNA IU/ml	Number of samples tested			HCV RNA IU/ml	Number of samples tested		
	HIV-1/HCV (duplex)	Discriminatory			HIV-1/HCV (duplex)	Discriminatory	
		HCV	HIV			HIV	HCV
250	24	8	7	250	24	6	6
100	34	8	8	100	34	6	6
50	34	8	7	50	34	6	5
20	34	8	8	20	34	6	5
10	24	8	8	10	24	6	2
5	24	6	4	5	24	4	0
2,5	24	5	5				

Table 2. HCV RNA concentration in dilutions of plasma samples with genotype 4c/4d and 3a

Tabela 2. Koncentracja HCV w rozcieńczeniach próbek osocza zakażonych genotypem 4c/4d i 3a

Genotype	Donation number	HCV RNA IU/ml concentration in dilutions tested
4c/4d	10-00-95111	5880, 588, 117, 29.25
	17-00-100552	1800, 180, 36,18
	12-02-127730	6730, 673, 134, 33.5
3a	06-00-69353	41600, 4160, 832, 41.6
	009280	39300, 3930, 786, 39.3
	010026	6460, 656, 129, 32.25

various concentrations of WHO HCV RNA and HIV RNA standards and 6 highly viraemic HCV "window period" donations (3.93×10^6–4.78×10^7 IU HCV RNA/ml).

Consecutive samples from blood donors with repeat reactive (RR) or indeterminate results of EIA anti-HCV (group I) or anti-HIV (group II) identified during blood donor screening between May 2003 and June 2004 were sent to the Reference Laboratory and tested for HCV RNA and HIV RNA. According to Polish regulations, Vacutainer EDTA tubes for molecular biology, with a gel barrier were used (Becton Dickinson, UK or Sarstedt, Germany). Group I consisted of 392 blood donors with anti-HCV RR EIA 3.0 (Ortho-Clinical Diag; S/C: >1) and 35 within "grey zone" results (S/C: 0.725–0.99). Group II consisted of 557 blood donors with anti-HIV screening EIA RR results (BioMerieux, S/C: >1): 21 confirmed by Western Blot (WB) test, 244 with indeterminate WB results, and 292 negative in WB. Moreover, 79 EIA indeterminate (S/C: 0.8––0.99)/WB negative and 62 indeterminate in both EIA and WB plasma samples were tested.

Serological methods

Anti-HCV: EIA 3.0 (Ortho-Clinical Diag; USA), UBI HCV EIA 4.0,

Anti-HIV: EIA HIV Uni-Form II Ag/Ab (bioMerieux bv, Netherlands), Western Blot — New LAV Blot I (BIO-RAD, France).

Molecular methods

Procleix HIV-1/HCV Assay (Gen-Probe/Chiron, USA) for simultaneous detection of HIV-1 and HCV RNA was performed in 500 μl of plasma according to the manufacturer's instruction. To discriminate the presence of HCV RNA and/or HIV RNA, the initially reactive samples were tested by discriminatory HCV and HIV assays in 500 μl of plasma for each test. These tests were performed in selected HCV and HIV

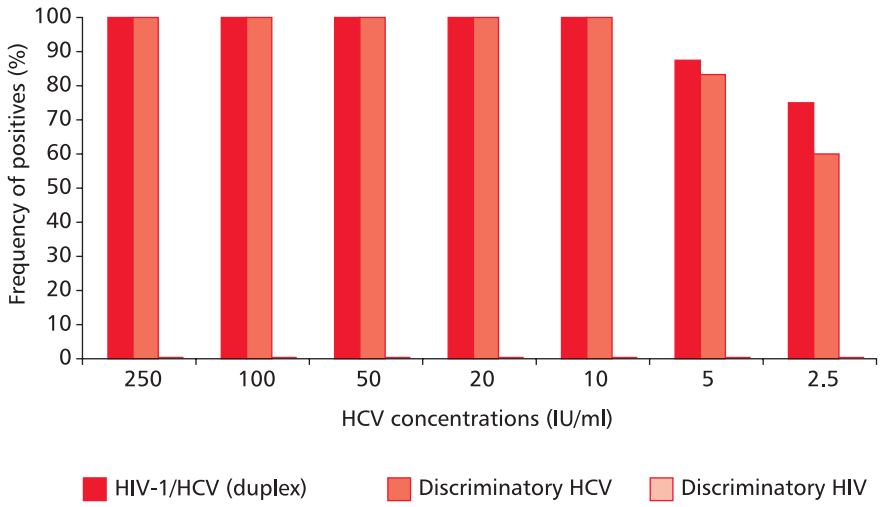

Figure 1. HCV RNA detection by Procleix HIV-1/HCV Assay in dilutions of HCV WHO standard

Rycina 1. Wykrywanie RNA HCV testem Procleix HIV-1/HCV w rozcieńczeniach standardu HCV WHO

positive standard (Table 1) and in all reactive blood donor samples. In the discriminatory assay, HIV-1 specific probe and HCV specific probe were used instead of the HIV-1 and HCV duplex probe reagent. The assay procedure was the same as in the duplex assay.

Results

Sensitivity of Procleix HIV-1/HCV assay (duplex assay)

All samples (150/150) containing at least 10 IU HCV RNA/ml (10, 20, 50, 100, and 250 IU HCV RNA/ml) were positive in the Procleix Assay (Figure 1). HCV RNA was detected in 39/48 samples containing 2.5–5 IU HCV RNA/ml. HIV RNA was detected in all samples (58/58) containing at least 100 IU HIV RNA/ml, in all but one (33/34) standards with 50 IU HIV RNA/ml, in 24/34 standards with 20 IU HIV RNA/ml, and in 28/48 samples containing 5–10 IU HIV RNA/ml (Figure 2). Probit analysis predicted 95% detection of HCV and HIV at 6.2 IU/ml and 44.5 IU/ml, respectively, and 50% detection limit at 1.5 IU/ml and 6.8 IU/ml, respectively (Table 3).

HCV genotype detection

HCV Genotypes 3 and 4 were detected in all dilution repetitions including the lowest concentrations tested (18 IU/ml for 4c/d and 32 IU/ml for 3a).

Specificity

None of the 291 anti-HCV/anti-HIV/HCV RNA negative samples tested simultaneously with various concentrations of WHO HCV RNA and HIV RNA standards and with 6 highly viremic HCV "window period" donations (3.93×10^6–4.78×10^7 IU/ml) was found positive. The frequency of invalid results was 1/813 (0.12%).

HIV and HCV nucleic acid detection in blood donors with different EIA results

HIV RNA was detected in all 21 anti-HIV EIA positive/WB positive blood donors (Table 4). We did not find any HIV RNA positive samples among donors with EIA positive results not confirmed by Western Blot (WB negative and indeterminate results, n = 536) or in any EIA indeterminate (n = 141) samples (Table 4). In 4 donors positive for anti-HIV by EIA and WB and for HIV RNA by Procleix, HCV RNA was also detected (all were anti-HCV positive as well).

HCV RNA was detected in 71/392 (18.1%) anti-HCV EIA positive plasma samples (Table 5). Correlation was observed between EIA S/C ratio and HCV RNA detectability: HCV RNA was detected in 68/105 (64.8%) of donors at ratio > 4, in 1/85 (1.2%) with S/C between 2.00 and 3.99, and in 2/202 donors (1%) with S/C between 1.00 and 1.99 Procleix HIV-1/HCV Assay results were negative in all donors within "grey zone" (S/C ratio: 0.725––0.99) and in all those in the control group.

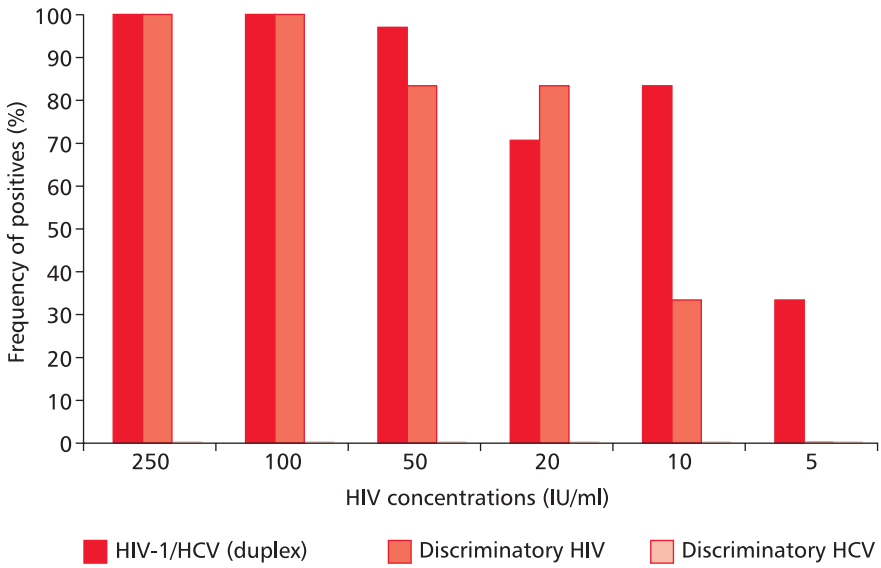

Figure 2. HIV RNA detection by Procleix HIV-1/HCV Assay in dilutions of HIV WHO standard

Rycina 2. Wykrywanie RNA HIV testem Procleix HIV-1/HCV w rozcieńczeniach standardu HIV WHO

Table 3. Estimation of 50% and 95% analytical sensitivity of the Procleix HIV-1/HCV Assay with 95% fiducial intervals - Probit analysis results for HCV and HIV WHO Standards

Tabela 3. Analiza Probit wyników badania rozcieńczeń standardów HCV i HIV WHO – 50% i 95% czułość analityczna testu

Sample/test	50% level IU/ml	95% Fiducial interval (IU/ml)	95% level IU/ml	95% Fiducial interval (IU/ml)
HIV – Duplex	6.8	(0.7, 12.8)	44.5	(21.9, 1472)
HCV – Duplex	1.5	(0.2, 2.4)	6.2	(4.3, 22.5)
HIV–Discriminatory	14.6	(6.7, 25.2)	55.1	(30.0, 495.5)
HCV–Discriminatory	2.2		6.7	

Table 4. HIV RNA detection by Chiron TMA Assay in blood donor samples with different results in serological tests

Tabela 4. Wykrywanie RNA HIV testem Procleix HIV1/HCV w próbkach dawców krwi z różnymi wynikami badań serologicznych

Results of serological tests			HIV RNA detection by PROCLEIX HIV-1/HCV	
EIA S/C	Western Blot	Number tested	HIV RNA positives	
			Number	Frequency
>1	+	21	21*	100%
>1	IND	244	0	0%
>1	–	292	0	0%
0.80–0.99	IND	62	0	0%
0.80–0.99	–	79	0	0%

*HIV/HCV S/C: 18.88–35.78; HIV S/C: 8.01–26.94, in 4 HCV RNA was also detected: HIV/HCV S/C: 29.23–35.78, HIV S/C: 22.03–26.62, HCV S/C: 18.55–23.83

Table 5. HCV RNA detection by Chiron TMA Assay in blood donor samples with different results in serological tests

Tabela 5. Wykrywanie RNA HCV testem Procleix HIV1/HCV w próbkach dawców krwi z różnymi wynikami testów serologicznych

Anti-HCV EIA			HCV RNA detection by PROCLEIX HIV-1/HCV	
Result	S/C range	Number Tested	HCV RNA positives	
			Number	Frequency
"Grey zone"	0.725–0.99	35	0	0 %
"Weak" positive	1–1.99	202	2*	1%
Positive	2–3.99	85	1**	1.2%
"Strong" Positive	> 4.00	105	68***	64.8%

*TMA HCV/HIV – S/C: 9.95–10.11, TMA HCV – S/C: 22.01; **TMA HCV/HIV – S/C: 11.18, TMA HCV – S/C: 22.20; ***TMA HCV/HIV – S/C: 9.00–12.53, TMA HCV – S/C: 20.95–25.10

Discussion

The analytical sensitivity of the TMA based assay is similar to the RT-PCR based assay. The 95% sensitivity of the Roche HCV Amplicor ranges from 16 to 25 IU/ml depending on the isolation applied [4]. For the Cobas Ampliscreen HIV-1 test, it is 78.4 IU/ml [5]. For the TMA based assay used in the present study, the 95% detection limit was 6.2 IU HCV RNA/ml and 44.5 IU HIV RNA/ml. These results comply with the requirements of the Committee for Proprietary Medical Products (CPMP/BWP/390/97, 1998) of the Council of Europe (100 IU/ml ~ 400 genomes equivalents per ml) [6].

HCV high variability and different genotype distributions were reported by many authors worldwide [7]. We confirm the high sensitivity of the Chiron test for genotypes 1b, 3a, and 4, most frequently detected in HCV infected Polish blood donors [8].

The aim of the second part of the study was the analysis of the usefulness of Procleix for the confirmation of active infection in blood donors with positive results in serological tests. Our observations are similar to those of others who confirmed HCV infection in no more than 30% of donors repeated reactive in screening viral enzyme immunoassays [9, 10]. Some other authors, however, observed higher HCV confirmation rates in low risk populations (75% — [11]).

We noticed that an S/C value of anti-HCV > 4 is a good predictor of HCV RNA detection since the frequency of positive results of HCV RNA was 64.8% in this group. This was confirmed by others, who underline that the low-positive anti-hepatitis C virus enzyme immunoassay result is a predictor of low likelihood of hepatitis C infection [12]. In our study, only 1.0% (3/287) of donors with S/C ratio 1–3.99 was infected.

It is difficult to interpret the anti-HCV positive/RNA negative results but we cannot entirely exclude that such donors are infected. The fluctuation of HCV RNA detectability in plasma and, the presence of HCV RNA in mononuclear cells but not in plasma have been described [13, 14]. For the safety of blood transfusions, such individuals should be treated as potentially infectious [14]. Such a marker pattern is also characteristic for individuals who have recovered from HCV infection. The frequency of spontaneous recovery is generally low; it is estimated at 24–46% [15, 16]. The number of prospective studies of such cases is still rather small. In Poland, between January 2000 and December 2003, we identified 50 donors in the "window period". All of them seroconverted in the follow-up study; 2 of 4 observed for >12 months, spontaneously cleared HCV RNA, but not anti-HCV [8].

The specificity of EIA for anti-HCV should also be considered. The reasons for false positivity of serological tests have been widely discussed in literature. The changes in the EIA reactivity are connected with lab-to-lab variation due to different equipment, different technicians, and different master lots used [17]. Vaccinations such as influenza or rabies, acute infections and allergies, immunologically related agents, such as transplantation antigens, auto- and heterophile antibodies, and reactivity to cellular and nondiagnostic proteins in the antigen preparation, were reported to be associated with false-positive screening test results [17, 18].

The low frequency of active infections among anti-HCV positive blood donors might also be connected with the implementation of NAT for blood

donation screening in 2000. At present, by screening for HCV RNA, we select all HCV infected blood donors before they become anti-EIA positive and we remove them from the registry. Thus, we reduce the number of HCV RNA positive donors among the EIA RR regular blood donors.

The frequency of EIA false positive results (536/557 — 96.2%) observed in anti-HIV EIA positive donors is even higher than anti-HCV EIA (321/ /392 — 81.9%). It is worth underlining that our study showed full concordance between WB tests results and the confirmation of HIV infection by molecular methods: HIV RNA was detected in all WB positive samples and in none of the WB negative ones. The reason for the high frequency of false positivity of anti-HIV EIA tests is not known. Ownby observed that anti-HIV false positive result prevalence was higher among women [17]. The cross-reactive antibodies resulting from alloimmunization during pregnancy may contribute to the difference. Some authors reported a trend toward declining prevalence with age [17]. In our study, false positive (not confirmed in HIV RNA testing and in WB) anti-HIV positive results were also two-fold more frequent among women than men but the differences were not statistically significant, probably due to the small number of female donors. Preliminary evidence suggests that low-risk subjects with indeterminate Western Blot might be infected with HIV-related viruses (other retroviruses) [19].

Our study shows that the HCV and HIV-1 TMA duplex had high specificity and sensitivity and can successfully be used to confirm infection in EIA positive individuals. It is still to be considered, if HCV RNA and HIV RNA testing might replace serological tests, as in the case of WB for anti-HIV and RIBA for anti-HCV. The second possibility, which might considerably reduce the costs of confirming infection, is to make the confirmation algorithm sequential with two EIA screening tests. According to Allain and Seed, it is less laborious, cheaper, and safe for blood donors [20, 10]. We also propose to introduce external uniform quality control of viral screening tests for all blood centres to limit the frequency of false positive results related to human error or equipment.

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