



## The use of complementary serological and molecular testing for blood-borne pathogens and evaluation of socio-demographic characteristics of intravenous drug users on substitution therapy from Šumadia district of Serbia

Komplementarno serološko i molekularno testiranje krvno-prenosivih patogena i procena sociodemografskih karakteristika kod korisnika intravenskih droga na supstitucionoj terapiji u Šumadijskom okrugu Srbije

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### Abstract

**Background/Aim.** Intravenous drug users (IDUs) are still a high risk-group for cross-reacting blood-borne infections, for vertical pathogen transmission as well as for potentially blood/plasma donation (especially as “paid” donors). The aim of our study was to establish the profile of opiate addict and prevalence of blood-borne pathogens – Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) among 99 patients on substitution therapy with methadone and buprenorphine from Šumadia District. **Methods.** The Treatment Demand Indicator (TDI) of Pampidou-questionnaire was used to assess the history of drug abuse and risk behavior. All blood samples were tested for HBV surface antigen (HBsAg), anti-HCV antibody (anti-HCV) and HIV antigen/antibody (HIV-Ag/Ab) by Enzyme-Linked Immunosorbent Assay (ELISA) or Chemiluminescent Immuno-Assay (CIA). Investigations were also performed for HBV, HCV and HIV by molecular testing – Polymerase Chain Reaction (PCR) method. **Results.** The majority of patients were males (81.8%), median age 32 (19–57) years, lived in a city (99%), unemployed (58.6%), with finished secondary school (67.7%), unsafe injecting practices (34.3%) and never previously tested for HBV (39.4%), HCV (36.4%) nor HIV (28.3%); only 4% of them previously got HBV-vaccine. The complementary testing resulted with following results: HBV

ELISA/CIA and PCR negativity for 66 patients and positive results (by ELISA/CIA and PCR) for 19 patients. However, a difference was observed in the ELISA/CIA-negative/PCR-positive result for 12 and ELISA/CIA-positive/PCR-negative for two patients respectively. Further, the negative results for HCV (ELISA/CIA and PCR testing) were found in 15 IDUs and positive results (using both methods) were found in 58 patients. Different results for ELISA/CIA-negative / PCR-positive results were found in 11 IDUs and ELISA/CIA-positive/PCR – negative results were found in 15 patients. All investigated IDUs were negative for HIV (ELISA/CIA and PCR testing) and for pathogens of opportunistic infection (*Cryptococcus neoformans*; *Pneumocystis carinii*; PCR testing), as well as for West Nile Virus (PCR testing). Just one IDU was positive for syphilis (ELISA and confirmatory testing). **Conclusion.** Our study demonstrated that the positivity for HBV and HCV is still very high (33.4% and 84.8%, respectively) in IDUs. Thus, we suggest that drug users have to be periodically screened using a complementary serological/molecular testing – concerning differences/discrepancies in the results obtained using these methods.

**Key words:**  
opioid-related disorders; blood-borne pathogens;  
methadone; hepatitis b virus; hepatitis c virus; hiv;  
serology; demography; serbia.

## Apstrakt

**Uvod/Cilj.** Korisnici intravenskih droga predstavljaju visokorizičnu grupu zbog međusobnih infekcija krvno prenosivim bolestima, vertikalne transmisije patogena, kao i zbog mogućnosti da budu potencijalni donori krvi/produkata plazme (naročito kao plaćeni donori). Cilj našeg istraživanja bio je utvrđivanje demografsko-sociološkog profila 99 opijatnih zavisnika Šumadijskog okruga lečenih u Kliničkom centru Kragujevac supstitucionom terapijom metadonom i buprenorfinom, kao i određivanje prevalencije infekcija krvno prenosivim bolestima: virusom hepatitisa B (HBV), virusom hepatitisa C (HCV) i virusom stečene imunodeficijencije (HIV). **Metode.** Ispitanici su odgovarali na pitanja iz Pompidu upitnika i podaci iz ovog upitnika korišćeni su za analizu osnovnih socio-demografskih karakteristika. Svi uzorci su prvo testirani ELISA (*Enzyme-Linked ImmunoSorbent Assay*) i CIA (*Chemiluminescent Immuno-Assay*) metodom, a zatim PCR-om (*Polymerase Chain Reaction*). **Rezultati.** Najveći broj ispitanika bilo je muškog pola (81,8%), starosti 32 (19–57) godine, 99% ispitanika živelo je u gradu, nezaposlenih je bilo 58,6%, sa završenom srednjom školom 67,7%, a korisnika neadekvatne primene igala bilo je 34,3%. Netestiranih na HBV bilo je 39,4%, na HCV 36,4%, HIV 28,3% a samo njih 4 (4%) primilo je vakcinu protiv HBV. Što se tiče analiza na prisustvo HBV infekcije, ELI-

SA/CIA i PCR negativnih je bilo 66, HBV ELISA/CIA i PCR pozitivnih bilo je 19, HBV ELISA/CIA-negativnih/PCR-pozitivnih 12 i HBV ELISA/CIA-pozitivnih/PCR-negativnih 2 ispitanika. Testiranje na HCV infekciju je pokazalo sledeće: ELISA/CIA i PCR negativnih ispitanika je bilo 15, HCV ELISA/CIA i PCR pozitivnih bilo je 58, HCV ELISA/CIA-negativnih/PCR-pozitivnih 11, a HCV ELISA/CIA-pozitivnih/PCR-negativnih 15. Svi ispitanici bili su negativni na HIV (ELISA/CIA i PCR testiranje), kao i na patogene oportunističkih infekcija (*Cryptococcus neoformans*; *Pneumocystis carinii*; PCR testiranje) i na prisustvo virusa zapadnog Nila (West Nile Virus; PCR testiranje). Jedan ispitanik bio je pozitivan na sifilis (ELISA testiranje). **Zaključak.** Naši rezultati pokazali su da je pozitivnost na prisustvo patogena krvno prenosivih bolesti HBV i HCV visoka u ispitivanoj grupi korisnika intravenskih droga i iznosi 33,4% i 84,8%, respektivno. Preporuka bi bila da oni budu periodično testirani na prisustvo HBV, HCV i HIV infekcije komplementarnim ELISA/CIA testovima, kao i PCR testovima, obzirom na izvestan stepen diskrepance u dobijenim rezultatima serološkog i molekularnog testiranja.

### Ključne reči:

**poremećaji izazvani opioidima; krvno-prenosivi patogeni; metadon; hepatitis b, virus; hepacivirus; hiv; serologija; demografija; srbija.**

## Introduction

The integrative approach is the necessity in the modern treatment of addiction, considering especially an early detection and additional treatment of somatic states in intravenous drug users (IDUs)<sup>1</sup>. The “drug-use-disorder” show to be very frequent in European countries (approximately 0.5% of population or about two million people), with relatively more problems of diseases caused by “drug-use-disorder” in Western Europe and especially high rates of hepatitis B virus (HBV), hepatitis C virus (HCV) and humane immunodeficiency virus (HIV) infection in this vulnerable population<sup>2,3</sup>.

The intravenous drug users face stigma not only due to psychological and behavioral aspects of their functioning, but also because of significantly higher rates of blood-borne and/or sexually transmitted infections due to unsafe injecting practices and risky sexual behaviors<sup>4</sup>. The pharmacological substitution programs of methadone and buprenorphine are the “harm avoidance” programs that are also useful in prevention of blood-borne infections<sup>5</sup>. The opiate addiction treatment in Serbia is conducted in four clinical centers by supervision of the Ministry of Health of the Republic of Serbia – including more than 4,000 patients on substitution therapy, but objective, precise and longitudinal data about opiate addiction and infectious disease “co-occurrence” is still missing<sup>6</sup>.

The serological testing, like routine screening of blood donors, are performed by the anti-HCV Enzyme Linked ImmunoSorbent Assay (ELISA) or Chemiluminescent Immuno-Assays (CIA) methods. In the last years of the 20th century, two more tests were initiated to detect the presence of HCV: HCV Ag/Ab (antigen/antibody), and HCV Nucleic

Acid Testing (NAT) or Polymerase Chain Reaction (PCR) assays<sup>7,8</sup>.

The period of the “window” (the time from entering the virus in the body until the moment when it is detectable by the available techniques) before the introduction of PCR was about 70 days. By introducing the PCR individual testing, this period is reduced to 15 days<sup>2</sup>, while the window period for HCV Ag/Ab is 40 days<sup>7</sup>. The window period is 16 days for HIV Ag/Ab, while in HIV PCR it is reduced to 9 days<sup>7</sup>. The actuality of comparisons of test results by the ELISA and PCR methods lies in determining the infectivity of the samples or the phase of infection in which there are dependents on psychoactive substances in this case<sup>3</sup>. It is necessary to determine the infectivity of the tested addicts and in terms of delineation of the test results: they did not come into contact with these viruses, the start of the infection – the “window” period, an infection, and past active infection.

The study aim was to evaluate the profile of an opiate addict and above all the prevalence of blood-borne infections such as HBV, HCV and HIV among IDUs on substitution therapy with methadone and buprenorphine in Šumadia District of Serbia. The results of complementary ELISA or CIA and PCR testing were also compared in attempt to improve the pathogen monitoring system as well as the diagnostic algorithm for this vulnerable population.

## Methods

### Patients

In this study the patients on substitution therapy with methadone or buprenorphine at the Department of Addicti-

ons, Psychiatric Clinic, Clinical Centre Kragujevac – as a regional addiction treatment center – were included. This centre is managing the drug dependence treatment using the Treatment Demand Indicator (TDI) approach. The TDI was formulated in 2000 by the European Monitoring Centre for Drugs and Drug Addiction – EMCDDA/Pompidou Group, aiming to collect comparable and reliable data about the number and characteristics of drug addicts in EU countries<sup>9</sup>.

TDI evaluates the treatment needs and assesses the history of drug abuse and risk behavior. Collected data from this questionnaire was used for the socio-demographic and injection practice analysis. Diagnoses of opioid related disorders (F11) or other psychoactive substance related disorders (F19) were established using the International Statistical Classification of Diseases and Related Health Problems.

The protocol of this “cross-sectional” study was approved by the Ethics Committee of the Clinical Centre Kragujevac and conducted in accordance with all the ethical principles of the Declaration of Helsinki. Informed consent was obtained from all of the patients before starting any study procedure.

#### Pathogen investigation methods

Total of 99 IDUs were tested using the complementary serological and molecular testing. All samples were taken into 9 mL tubes with K2EDTA (Bio-One Vacuette, Greiner), then the tubes were centrifuged at 3500 rpm, 30 minutes and plasma samples were analyzed. The samples were initially tested by ELISA or CIA systems (Evolis, Biorad; Architect i2000 SR, Abbott) and afterward with s201 system (COBAS Ampliprep/ COBAS Taqman, Roche). The preliminary positive specimens were analyzed using ID (Individual Donation) PCR. The preliminary negative samples were tested by mini-pool (MP) PCR technique (6 samples).

HCV ELISA/CIA-negative/PCR-positive samples were tested using the confirmatory test (n = 11; Innolia Innogenetics)<sup>7</sup>. In addition, all 99 IDUs were tested on pathogens of opportunistic infections, such as *Cryptococcus neoformans* and *Pneumocystis carinii*. Finally, the patients were also investigated for syphilis by ELISA or CIA method and West Nile Virus (WNV) by the PCR testing.

The pathogen investigations were performed in the Institute of Transfusiology and Hemobiology of the Military Medical Academy (MMA) and in the Institute of Microbiology of the MMA in Belgrade – during the period from July to August 2015.

#### Statistical analysis

The data was presented as absolute numbers, median and percentage. The tables were used to present the socio-demographic characteristics of IDUs as well as the complementary serological and molecular investigations. The 2 × 2 contingency table was used to compare the results of serological and molecular testing. The statistical analyses were performed using the SPSS 20.0 software. Differences were considered as statistically significant if the *p* value was less than 0.05.

#### Results

The majority of IDUs were males, city residents and prevalingly had completed the secondary school (their characteristics are presented in Table 1).

**Table 1**

#### The socio-demographic characteristics of IDUs

Parameters	Values
Age (years)	
median	32
range	19–57
Gender, n (%)	
male	88 (81.8)
female	11 (18.2)
Residents of the city, n (%)	98 (98.9)
Unemployed, n (%)	58 (58.6)
Education level – secondary school, n (%)	67 (67.7)
Unsafe injecting practices, n (%)	34 (34.3)

#### IDUs – intravenous drug users.

Amongst all 99 IDUs, some of them were never tested on virus infections – exactly, on HBV 39, on HCV 36, on HIV 28; in addition, just 4 IDUs got a vaccine against HBV.

The complementary ELISA or CIA and PCR testing of the IDUs demonstrated the predominant concordance between the serological and molecular analysis. The results confirmed that more than 80% of IDUs had HCV positivity, proved by both testing, comparing to more than 30% proved HBV infection. The concordance and discordance between the different methods (ELISA or CIA vs. PCR) are shown in Table 2.

Absolute numbers of patients – analyzed by the comparative serological and molecular testing for HCV vs. HBV in this study – are summarized in Table 3 and Figure 1.

**Table 2**

#### Complementary ELISA or CIA and PCR investigations for viruses

Pathogen type	ELISA or CIA and PCR testing	IDUs n (%)
HBV	ELISA/CIA-negative/PCR-negative	66 (66.7)
	ELISA/CIA-positive/PCR-positive	19 (19.2)
	ELISA/CIA-negative/PCR-positive	12 (12.1)
	ELISA/CIA-positive/PCR-negative	2 (2.1)
HCV	ELISA/CIA-negative/PCR-negative	15 (15.1)
	ELISA/CIA-positive/PCR-positive	58 (58.6)
	ELISA/CIA-negative/PCR-positive	11 (11.1)
	ELISA/CIA-positive/PCR-negative	15 (15.1)
HIV	ELISA/CIA-negative/PCR-negative	99 (100)

#### HIV – human immunodeficiency virus.

For other abbreviations see under Figure 1.

Table 3

## The HBV and HCV presence investigated by ELISA or CIA and PCR

ELISA or CIA testing (absolute numbers)			PCR testing (absolute numbers)		
Pathogen type	HCV		Pathogen type	HCV	
	negative	positive		negative	positive
HBV			HBV		
negative	25	56*	negative	18	50*
positive	1*	17*	positive	12*	19*

\*significant difference in the HBV and HCV positivity (1 + 17 vs. 56+17; 12+19 vs. 50 + 19);  $p < 0.01$ .

For abbreviations see under Figure 1.

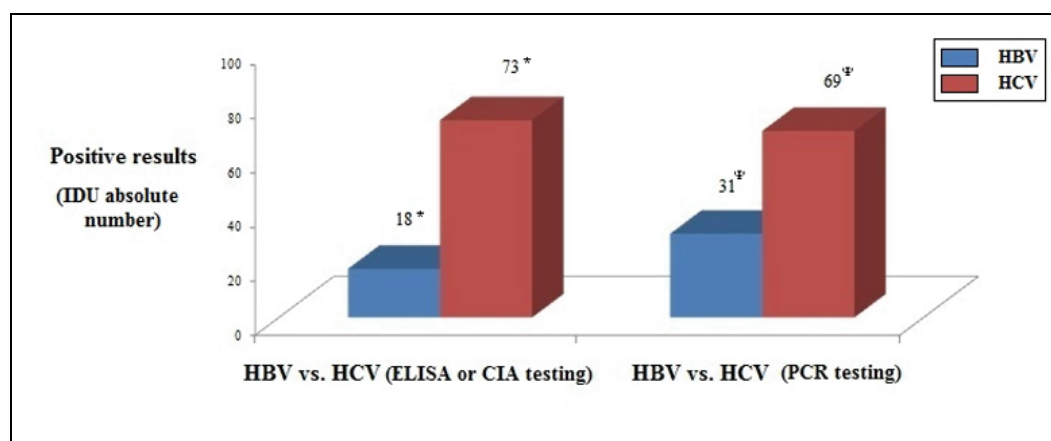


Fig. 1 – Comparative HCV and HBV values determined by ELISA/CIA and PCR.

\*,\*<sup>‡</sup> significant differences between HBV and HCV positivity by both, serological and molecular testing ( $p < 0.01$ ). IDU – intravenous drug users; HCV – hepatitis C virus; HBV – hepatitis B virus; ELISA/CIA – Enzyme-linked Immunosorbent Assay/Chemiluminiscent Immuno Assay; PCR – Polymerase Chain Reaction.

Table 4

## The results of ELISA or CIA and PCR testing for other pathogens

Pathogen type	ELISA or CIA and PCR testing	IDU n (%)
<i>Treponema pallidum</i>	ELISA/CIA-negative	98 (99)
	ELISA-positive	
	*VDRL-positive	1 (1)
	**TPHA-positive	
	Confirmatory test-positive	
<i>Cryptococcus neoformans</i>	PCR-negative	99 (100)
<i>Pneumocystis carinii</i>	PCR-negative	99 (100)
West Nile Virus	PCR-negative	99 (100)

IDU – intravenous drug users; VDRL – Venereal Disease Research Laboratory testing; TPHA – *Treponema pallidum* Hemagglutination test.

For other abbreviations see under Figure 1.

As presented, the number of HCV vs. HBV positive IDUs was significantly ( $p < 0.01$ ) higher in our study group, using both ELISA or CIA and PCR techniques.

Regarding the opportunistic infections, all 99 IDUs were negative (PCR testing) for the most common pathogens: *Cryptococcus neoformans*, *Pneumocystis carinii* and WNV. Only one patient was positive on syphilis by ELISA, VDRL and TPHA. These results are presented in Table 4.

The presented negativity for the opportunistic infection pathogens can be explained because of no severe compromised immune system – since all IDUs were negative on HIV.

## Discussion

Several risk factors make IDUs vulnerable to HCV, HBV, HIV, syphilis and opportunistic infection caused by *Cryptococcus neoformans*, *Pneumocystis carinii* and WNV<sup>10</sup>. Hazardous behaviors include the use of non-sterilized needles and unprotected sexual activities, unsafe tattooing, cupping, blood transfusion or dental procedures in both IDUs and non-IDUs. Besides, a lack of access to health services, low socio-educational level, homelessness, history of imprisonment, social exclusion, unemployment, alcohol addiction

and presence of other diseases complicate the features of infection by HCV, HBV and HIV viruses and their related outcomes in many IDUs.

The global prevalence of HCV infection among IDUs in 2010 was 46.7%, implicating that some 7.4 million of the 16 million IDUs worldwide are infected with the HCV. The HBV infection rate among IDUs is about 14.6%, that is 2.3 million IDU are infected with mentioned virus, and 18.9% or 3 million of IDUs are living with HIV worldwide<sup>11</sup>.

Despite the higher prevalence and “transmissibility” and the equal or higher economic costs of HCV compared to HIV infection, especially among IDUs, viral hepatitis received far less attention than HIV related disease. Worldwide, the prevalence of HIV infection amongst IDUs was calculated as 17.9% in 2009 and 18.9% in 2010<sup>3</sup>.

In our study there is no HIV infection among IDUs and that is similar as prevalence in Iran (0.7%) and among blood donors tested earlier in MMA (0.005%)<sup>8,12</sup>. Prevalence of HBV (33.4%) was lower than in Italy (where the prevalence is 60.7%), while in Mexico it is 85%, similar as in Greece and Portugal, but significantly higher than prevalence in Uruguay (20%), Iran (0.7%) and among blood donors (0.20%)<sup>13–15</sup>. IDUs had a much higher probability of acquiring infection than non-injectors, confirming the role of intravenous transmission. The prevalence was highest for HCV infection (84.8%) and that is lower than prevalence observed in Estonia and Latvia (about 90%), Romania and Portugal, and similar as in Russia (73%). The HCV prevalence is higher than in Hungary (23%) and among blood donors (0.12%)<sup>12–16</sup>.

The difference between results of ELISA/CIA and PCR testing can be explained in two ways. Firstly, ELISA/CIA negative, but PCR positive results show that infection with HBV or HCV is in the “window” period – that means that the concentration of viral antigen or antibodies against them are too low that they cannot be measured by ELISA/CIA<sup>8</sup>. On the other hand, the PCR negative ELISA/CIA positive results are common when we have cases of old HCV infections. The number of these results can be even 20% among

blood donors, so the prevalence of 15.1% in our study is in that range<sup>17,18</sup>. The number of two HBV PCR negative ELISA/CIA positive results show that the HBV DNA levels in the HBsAg-positive samples can be extremely low. About 6% of donations would be negative by the current MP HBV PCR methods. About 3% of donations would remain undetected by sensitive single-donor PCR. These results indicate caution in any consideration of dropping the HBsAg screening<sup>18</sup>.

IDUs in Serbia have similar social-demographic characteristics as in Italy: 88% vs. 84% are males, median age is 32 vs. 35 years, mostly unemployed. However, IDUs in this investigation were not well-educated because only 4% of patients received HBV vaccine – while 29% in Italy<sup>13</sup>. This fact could be a consequence of the suboptimal medical care system in investigated region.

### Conclusion

The majority of IDUs were males (aged 19–57 years), city residents and predominantly with completed secondary school. This study undoubtedly demonstrated improved safety of originally designed the complementary (ELISA/CIA and PCR) pathogen monitoring system. Our results confirmed that injecting drug practice continues to be an important risk factor for blood-borne infections; the positivity for HBV and HCV was still very high – 33.4% and 84.8%, respectively. Thus, drug users have to be periodically screened by the complementary serological/molecular testing – concerning differences/discrepancies in the results obtained by using these methods. Finally, we speculate that HBV vaccination should be actively obtainable/offered to all HBV-negative IDUs.

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### R E F E R E N C E S

1. *Wiessing L, Ferri M, Grady B, Kantzanou M, Sperle I, Cullen KJ, et al.* Hepatitis C virus infection epidemiology among people who inject drugs in Europe: a systematic review of data for scaling up treatment and prevention. *PLoS One* 2014; 9(7): e103345.
2. *Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B, et al.* The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol* 2011; 21(9): 655–79.
3. *Wales N.* A review of viral hepatitis in injecting drug users and assessment of priorities for future activities. Geneva: WHO; 2009.
4. *Gyarmathy VA, Neaigus A, Li N, Ujhelyi E, Caplinskiene I, Caplinskias S, et al.* Infection disclosure in the injecting dyads of Hungarian and Lithuanian injecting drug users who self-reported being infected with hepatitis C virus or human immunodeficiency virus. *Scand J Infect Dis* 2011; 43(1): 32–42.
5. *Ruan Y, Liang S, Zhu J, Li X, Pan SW, Liu Q, et al.* Evaluation of harm reduction programs on seroincidence of HIV, hepatitis B and C, and syphilis among intravenous drug users in southwest China. *Sex Transm Dis* 2013; 40(4): 323–8.
6. *Djukić Dejanović S, Borovčanin M.* Principles of treatment of opiate dependents in Serbia. In: *Djukić Dejanović S, Nastasić P, editors.* Dependence diseases: Modern achievements in Prevention, treatment and rehabilitation Beograd: ECPD; 2015. p. 10–2. (Serbian)
7. *Trkaljić M, Borovčanin N, Vučetić D, Jovičić D.* Transmissible diseases – Etiopathogenesis, markers testing, inactivation of pathogens. In: *Balint B, Trkaljić M, Todorović M, editors.* Basic principles of chemotherapy. Beograd: Čigoja štampa; 2010. p. 421–505. (Serbian)
8. *Vučetić D, Kecman G, Ilić V, Balint B.* Blood donors' positivity for transfusion-transmissible infections: the Serbian Military Medical Academy experience. *Blood Transfus* 2015; 13(4): 569–75.

9. *Simon R, Donmall M, Hartnoll R, Kokkevi A, Ouwehand AW, Stauffacher M, et al.* The EMCDDA/Pompidou Group treatment demand indicator protocol: a European core item set for treatment monitoring and reporting. *Eur Addict Res* 1999; 5(4): 197–207.
10. *Allain PJ.* Emerging Viruses in Transfusion. In: *Barbara JA, Regan FA, Contreras MC*, editors. *Transfusion Microbiology*. New York: Cambridge University Press; 2008. p. 75–86.
11. *Honarvar B, Odoomi N, Moghadami M, Kazerooni PA, Hassanabadi A, Dolatabadi PZ, et al.* Blood-Borne Hepatitis in Opiate Users in Iran: A Poor Outlook and Urgent Need to Change Nationwide Screening Policy. *PLoS One* 2013; 8(12): e82230.
12. *Zamania S, Radfarb R, Nematollahi P, Fadaiee R, Meshkati M, Mortazavia S, et al.* Prevalence of HIV/HCV/HBV infections and drug-related risk behaviours amongst IDUs recruited through peer-driven sampling in Iran. *Int J Drug Policy* 2010; 21(6): 493–500.
13. *Camoni L, Regine V, Salfa MC, Nicoletti G, Canuzzi P, Magliocchetti N, et al.* Continued high prevalence of HIV, HBV and HCV among injecting and noninjecting drug users in Italy. *Ann Ist Super Sanità* 2010; 46(1): 59–65.
14. *Gonlao J, Götz W.* European Monitoring Centre for Drugs and Drug Addiction. Annual Report 2012: The state of the drug problem in Europe. Lisbon: EMCDDA; 2012.
15. *Amon JJ.* Hepatitis in drug users: time for attention, time for action. *Lancet* 2011; 378 (9791): 543–4.
16. *Balint B, Vucetic D, Todorovic-Balint M, Borovcanin N, Jovanovic-Cupic S, Mandusic V.* Safety improving by complementary serological and molecular testing combined with pathogen reduction of the donated blood in window period (Letter). *Transfus Apher Sci* 2013; 49(1): 103–4.
17. *Busch M, Glynn S, Stramer S, Orland J, Murphy E, Wright D, et al.* Correlates of hepatitis C virus (HCV) RNA negativity among HCV- seropositive blood donors. *Transfusion* 2006; 46(3): 469–75.
18. *Kubns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP.* Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. *Transfusion* 2004; 44(9): 1332–9.

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