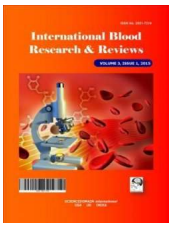

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External Quality Assessment of Transfusion-transmissible Infections Testing

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the overall performance of the transfusion-transmissible infection testing laboratory through the evaluation of the results obtained from the participation in a blood proficiency testing study (B-PTS).
Study Design: The B-PTS study was designed, organized and conducted by European directorate for the quality of medicines (EDQM). We were requested to test the B-PTS samples and to report the results on the online result data sheet.
Place and Duration of Study: The 3 blood testing laboratories of the Institute of transfusion medicine in Macedonia; July 2017.
Methodology: Each set of B-PTS-samples contained 4 panels: Anti-HCV (032), anti-HIV/p24 (033), anti-Treponema (034) and HBsAg panel (035). The samples were subjected to serological testing with two assays: Enzyme immunoassay with Enzygnost system, Siemens using BEP2000 and chemiluminescent microparticle immunoassay with Architect system, Abbott using Architect i2000.
Results: The laboratories were classified as "satisfactory" for B-PTS032 and B-PTS034. For B-PTS033 the classification was "non evaluable" because the results were not properly submitted. The B-PTS035 results were classified as "unsatisfactory" because two laboratories reported the reactive sample number 3 as "Not Reactive" with the Enzygnost assay and one laboratory reported it as "Not

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Reactive" with the Architect assay. The single observed non-conformity was that the S/Co (1.22) of the positive control for the Architect HBsAg assay was out of rang (1.65-4.96) for the corresponding reagent lot.
Conclusion: The participation in a B-PTS study provides an objective and independent evaluation of the overall performance of the laboratory. The management of the non-satisfactory PTS results should be documented and performed in a controlled manner. Appropriate corrective and preventive measures should be taken in order non-conformities not to repeat.

Keywords: Transfusion-transmissible infection; external quality assessment; blood proficiency testing.

ABBREVIATIONS

TTI: Transfusion-transmissible infection; EIA: Enzyme immunoassay; CMIA: Chemiluminescent microparticle immunoassay; NAT: Nucleic acid testing; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; EDQM: European directorate for the quality of medicines; EQA: External quality assessment; B-PTS: Blood proficiency testing scheme.

1. INTRODUCTION

According to the World Health Organization (WHO) the strategy concerning blood transfusion should be the policy of self-sufficiency, adequacy and safety of the blood supply. Safe blood starts with the donor and there is a general agreement that donors should be voluntary and non-remunerated. Along with the donor selection, laboratory screening of donated blood for transfusion- transmissible infection (TTI) markers is a key safety measure in protecting patients and preventing the spread of such infectious diseases in the community.

Depending on the epidemiological and economic situation, different technologies such as enzyme immunoassay (EIA), chemiluminescent microparticle immunoassay (CMIA) and recently nucleic acid testing (NAT) have been employed in different countries, as well as different panel of TTI markers. Screening of donated blood for TTI such as hepatitis B and C virus and human immunodeficiency virus (HIV) is recommended as a routine and is considered mandatory in most of the countries world-wide [1].

In order to improve safety of labile blood products and blood derived medicinal products and of patients undergoing blood transfusion, European directorate for the quality of medicines (EDQM) has implemented a proficiency testing scheme (PTS) programme starting from the year of 2013. Blood proficiency testing studies (B-PTS) are specially designated for use in blood transfusion laboratories as a method for measurement of the performance of laboratories, based on inter-laboratory comparison. Participation of the laboratories which perform TTI testing in external quality assessment (EQA) programmes such as B-PTS studies is an important factor for the quality assurance of blood products [2,3]. It provides laboratories with an objective means to assess and demonstrate the reliability of their data and the integrity of their entire testing process in order to identify sources of errors and to prevent erroneous results [4].

In July 2017, for the first time, three TTI testing laboratories form the Institute of transfusion medicine in Macedonia took place in the B-PTS study organized by EDQM. The aim was to assess the overall performance of the laboratory from the receipt and storage of the blood samples, throughout the performance of the testing of individual blood donations and to the final interpretation of the data. Thus, we report the results regarding the serologic testing of HBsAg, anti-HIV/p24, anti-HCV and anti-Treponema performed on B-PTS samples provided by EDQM, as well as the the outcome of the root-cause analysis of the non-satisfactory B-PTS results.

2. MATERIALS AND METHODS

2.1 Study Design, Duration and Setting

The B-PTS study was designed, organized and conducted by EDQM on behalf of the Council of Europe's European Committee on Blood Transfusion in the period from June to July 2017. More than 70 laboratories from 23 European blood establishments took part. Participation was on a voluntary basis, subsequent to prior online registration. The Institute of transfusion medicine

of Macedonia participated with three laboratories located in Skopje, Bitola and Stip. Participants were requested to test samples of the panel in their established, routinely used assay and to report the results on the online result data sheet, together with the name of the assay used.

2.2 Sample Size

We received three sets of B-PTS samples containing 4 panels which were distributed to our laboratories. Anti-HCV panel (B-PTS032) was composed of 5 samples, coded from 1 to 5. Anti-HIV/p24 panel (B-PTS033) was composed of 6 samples, coded from 1 to 6. Anti-Treponema panel (B-PTS034) was composed of 4 samples, coded from 1 to 4 and HBsAg panel (B-PTS035) was composed of 7 samples, coded from 1 to 7.

Each sample contained 1.1 mL liquid/frozen material. Each panel included core positive, non-core positive and core negative samples for the corresponding marker (the composition of the panels was not known to the participants at the time of the performance of the testing). The panels were produced by an external producer, under the supervision from the quality assurance department of EDQM. The production and labeling were performed in accordance with the requirements for reference material producers laid down in the International organization for standardization (ISO) guide 34:2000.

2.3 Testing Technique

Each of the B-PTS samples was tested by each of the three laboratories (Skopje, Bitola and Stip) with two serological assays such as enzyme immunoassay (EIA) with Enzygnost system, Siemens using auto analyzer BEP2000 and chemiluminescent microparticle immunoassay (CMIA) with Architect system, Abbott using auto analyzer Architect i2000.

The laboratory testing is performed according to the manufacturer's instructions concerning the assay procedure, reagents, specimen collection and preparation for analysis. Assay calibration and daily quality control procedures to verify the calibration are performed according to the manufacturer's instructions as well.

The overall sensitivity and specificity of the used reagents for Architect assays (anti-HCV, Syphilis, Ag/Ab HIV combo and HBsAg Qualitative II), as well as for the Enzygnost assays (anti-HCV 4.0, Syphilis, HIV Intergral 4.0 and HBsAg 6.0) is shown in the each of the package insert instructions of the reagents.

2.4 Reporting the Results

Each laboratory provided the Signal/Cut-off (S/Co) ratios for Architect assays and Signal (O.D.) values for Enzygnost assays for each B-PTS sample as well as the interpretation of the results (R=Reactive, NR= Not Reactive, Inc.=Inconclusive or D=Doubtful). Results were reported to EDQM electronically on the online results data sheet, together with the name of the assay used.

2.5 Evaluation Criteria by EDQM

The laboratory was classified "satisfactory" if all core positive and core negative samples were correctly determined as "reactive" (R) and "non-reactive" (NR), respectively. The laboratory was classified as "unsatisfactory" if at least one of the core positives and the core negative samples is not correctly determined as R and NR, respectively.

3. RESULTS

The obtained results were interpreted as "Not Reactive" if the S/Co value of the sample was < 1.00 and as "Reactive" if it was ≥ 1.00 for Architect assays. For Enzygnost assays, the results were interpreted as "Not Reactive" if the Signal (O.D.) value of the sample was below the calculated cutoff and as "Reactive" if it was above the calculated cutoff except for the Enzygnost Syphilis assays for which the interpretation is the opposite.

We received the EDQM reports on B-PTS (S-032, S-033, S-034 and S-035) in September 2017. Each laboratory received a code number allocated randomly by the organizers of the study.

According to the reports the laboratories in Skopje, Bitola and Stip were classified as "satisfactory" for B-PTS032: Anti-HCV and B-PTS034: anti-Treponema panel as shown on Tables 1 and 2 respectively.

The non-core positive PTS-032 samples 1 and 2 might be found not reactive or reactive according to the EDQM evaluation.

Table 1. Results of the B-PTS032: Anti-HCV panel

EDQM PTS-032	Skopje		Bitola		Stip	
	A* S/Co 1.00	E** Cutoff 0.338	A S/Co 1.00	E Cutoff 0.391	A S/Co 1.00	E Cutoff 0.336
1-NR/R	**R**	**NR**	**R**	**R**	**R**	**NR**
	1.24	0.298	1.73	0.486	1.29	0.273
2-NR/R	**R**	**R**	**R**	**R**	**R**	**R**
	1.44	0.439	1.72	0.627	1.43	0.370
3-NR	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**
	0.08	0.022	0.10	0.073	0.07	0.014
4-R	**R**	**R**	**R**	**R**	**R**	**R**
	3.76	0.957	5.80	1.274	4.49	0.817
5-R	**R**	**R**	**R**	**R**	**R**	**R**
	4.39	1.093	6.07	1.432	4.64	0.980

** Architect assay (anti-HCV)*
*** Enzygnost assay (anti-HCV 4.0)*

Table 2. Results of the B-PTS034: Anti-Treponema panel

EDQM PTS-034	Skopje		Bitola		Stip	
	A* S/Co 1.00	E** Cutoff 1.370	A S/Co 1.00	E Cutoff 1.010	A S/Co 1.00	E Cutoff 1.114
1-R	**R**	**R**	**R**	**R**	**R**	**R**
	16.69	0.067	16.06	0.090	18.60	0.050
2-NR	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**
	0.05	1.992	0.05	1.862	0.04	1.920
3-R	**R**	**R**	**R**	**R**	**R**	**R**
	6.73	0.583	7.05	0.498	6.95	0.513
4-R	**R**	**R**	**R**	**R**	**R**	**R**
	4.41	0.446	4.44	0.480	4.78	0.436

** Architect assay (Syphilis)*
*** Enzygnost assay (Syphilis)*

For B-PTS033 panel the classification was “non evaluable” because the results for sample 6 were not properly submitted and were not included in the report. However, the obtained results by the three laboratories were in concordance with the evaluation criteria for satisfactory performance (Table 3).

The non-core positive PTS-033 sample 5 might be found not reactive or reactive according to the EDQM evaluation.

The B-PTS035: HBsAg test results were classified as “unsatisfactory” because two laboratories (Skopje and Stip) reported the reactive sample 3 as “Not Reactive” with the Enzignost assay and Bitola laboratory reported the reactive sample 3 as “Not Reactive” with the Architect assay. The results obtained by the laboratories are listed in Table 4.

The non-core positive PTS-035 sample 2 might be found not reactive or reactive according to the EDQM evaluation.

4. DISCUSSION

Nowadays blood transfusion is one of the safest medical procedures. Never the less, there is still residual risk of infectious disease transmission which depends on the prevalence of the microbial agents in the population of donors and the technology of testing. The residual risk per unit transfused is 1:1.000.000 for HIV, 1:390.000 for HCV, 1:200-500.000 for HBV [5,6].

Annually about 50.000 blood units are tested for TTI by the three laboratories of the Institute of transfusion medicine in Macedonia. There is a quality management system (QMS) in our institution and written standard operating

procedures (SOPs) which cover every step in the process of blood collection, blood testing and preparation of blood products. National regulations permit specifically trained technicians to perform transfusion related activities in blood service laboratories. Algorithm for repeat and confirmatory testing of the initially reactive blood units is in place. The haemovigilance network in the country is still in development but there is a tradition of reporting of the serious adverse transfusion reaction. Until now there was not a single report on TTI disease by the clinicians.

With about 2.3 million donations per year, since 1996, in the UK there were 30 confirmed incidents of transfusion-transmitted viral infections, involving a total of 37 recipients, with HBV being the most commonly reported proven viral TTI [7].

What we have learned from the participation in the B-PTS study which was our first experience with an external quality assurance programme. First of all we realized that we should document and report the non-satisfactory PTS results. Such results should be treated as non-conformity (NC) and must be carefully investigated for causative factors and fallowed by implementation of corrective and preventive actions to prevent reoccurrence [2,4].

Table 3. Results of the B-PTS033: Anti-HIV/p24 panel

EDQM PTS-033	Skopje		Bitola		Stip	
	A* S/Co 1.00	**E** Cutoff 0.280**	**A S/Co 1.00**	**E Cutoff 0.283**	**A S/Co 1.00**	**E Cutoff 0.200**
1-NR	**NR** 0.10	**NR** 0.05	**NR** 0.15	**NR** 0.056	**NR** 0.10	**NR** 0.08
2-R	**R** 7.87	**R** 3.00	**R** 8.39	**R** 3.00	**R** 8.23	**R** 3.00
3-R	**R** 4.13	**R** 2.78	**R** 4.49	**R** 3.00	**R** 4.25	**R** 2.756
4-R	**R** 10.5	**R** 2.54	**R** 11.52	**R** 2.975	**R** 12.96	**R** 2.277
5-NR/R	**NR** 0.83	**R** 0.84	**R** 1.03	**R** 1.172	**NR** 0.93	**R** 0.658
6-R	**R** 2.83	**R** 1.53	**R** 2.88	**R** 1.839	**R** 2.94	**R** 1.348

** Architect assay (Ag/Ab HIV combo)*
*** Enzygnost assay (HIV integral 4)*

Table 4. Results of the B-PTS035: HBsAg panel

EDQM PTS-035	Skopje		Bitola		Stip	
	A* S/Co 1.00	**E** Cutoff 0.081**	**A S/Co 1.00**	**E Cutoff 0.074**	**A S/Co 1.00**	**E Cutoff 0.064**
1-R	**R** 5.43	**R** 0.24	**R** 1.90	**R** 0.440	**R** 4.32	**R** 0.190
2-R/NR	**R** 1.36	**NR** 0.02	**NR** 0.49	**NR** 0.073	**R** 1.14	**NR** 0.01
3-R	**R** 2.29	**NR** 0.055	**NR** 0.88	**R** 0.140	**R** 2.04	**NR** 0.03
4-R	**R** 5.61	**R** 0.17	**R** 1.99	**R** 0.366	**R** 4.87	**R** 0.118
5/7-NR	**NR** 0.25/0.20	**NR** 0.01/0.009	**NR** 0.10/0.09	**NR** 0.02/0.009	**NR** 0.19/0.03	**NR** 0.01/0.006
6-NR	**NR** 0.22	**NR** 0.009	**NR** 0.09	**NR** 0.018	**NR** 0.06	**NR** 0.006

** Architect assay (HBsAg Qualitative II)*
*** Enzygnost assay (HBsAg 6.0)*

For that purpose we fallowed the established procedure for reporting of NC to the quality management department. There are pre-designated lists (documents) for non-conformity reporting, management (steps of investigation) and undertaken corrective measures.

The quality improvement programme was approved by the quality manager (QM) and was conducted to investigate the root-cause of the non-satisfactory results of the B-PTS study in which we participated. The programme consisted of three phases: 1) Look back at the laboratory documentation, 2) Retesting and additional testing if necessary, 3) Corrective and preventive measures.

Phase 1: The good record keeping practice enabled us to look back at the laboratory documentation at the time of B-PTS samples testing and to check the parameters of the pre-analytical, analytical and post-analytical data concerning documentation on maintenance and validation of the instruments, room temperature of the laboratory and refrigerators in which the reagents are kept, validation, calibration and quality control sample runs (the lists of results of the validation and calibration parameters, quality control run results) and the reagent lots which were used.

We noticed that the S/Co value of the positive control for Architect HBsAg Qualitative II assay obtained in Bitola laboratory was 1.22 which was lower than the expected S/Co rang 1.65-4.96 for the used reagent lot. This might be the causative factor for the non-conformant results for B-PTS035 panel. Looking at the original list from the instrument we noticed that the values of the results for the B-PTS035 samples (1-7) obtained with Architect assay (HBsAg Qualitative II) from Bitola laboratory were about three times lower in comparison with the other two laboratories for each sample from the panel respectively (Table 4).

The root-cause analysis revealed that the laboratory in Bitola did not check the non-conformant result of the control-run (the positive control was out of rang) of the HBsAg assay for the Architect system obtained on the day when B-PTS samples were tested which caused the non-conformant result on the B-PTS035 sample number 3. Validation criteria for the Architect HBsAg Qualitative II assay were not interpreted correctly by the laboratory. They did not perform additional calibration of the used reagent lot and another quality control run.

We also notice that the values of the results of all of the samples of B-PTS035 panel obtained with Enzygnost assay (HBsAg 6.0) from Skopje and Stip laboratory were about 2 times lower for each sample respectively in comparison with Bitola laboratory as shown in Table 4, although there was no significant difference in the calculated cutoff and the negative and positive controls were within the validation limit.

Phase 2: We performed two repeated testing of the B-PTS035 panel with the reagent lot and control lot which was included in the used reagent kit. The calculated cutoff was 0,072 in the first and 0.059 in the second testing. The B-PTS035 sample designated as number 3 which was initially tested as non reactive with Enzygnost assay (HBsAg 6.0), in the two repeated tests was detected and interpreted as reactive with O.D. value of 0.138 and 0.137 respectively. Concerning the Enzygnost HBsAg 6.0 assay we failed to identify the root-cause factor for the non-satisfactory PTS results although the analysis points to the variation of the negative control values from lot to lot, sometimes being much higher than the negative values of the tested samples although still within the validation criteria.

Our analysis of the possible causative factors for the non-satisfactory PTS results indicated non-conformant performance in the analytical phase although according to the literature data most errors throughout the laboratory working process occurred in the pre- or post-analytical phases, whereas a minority (13–32% according to the studies) occurred in the analytical phase [8].

Phase 3: As a corrective measure, additional training of the laboratory staff was organised in the presence of the manufacturer's representatives and the Quality manager of our Institution. We went through all the steps of the instructions for use. We also agreed that a revision of the SOPs should be done as soon as possible. As a preventive measure we informed the manufactures and ask them for additional check of the instruments, as well as the pre-defined validation and calibration criteria.

The costs of the above mentioned investigation can be measured by the cost of the reagents used to perform the retesting of the original B-PTS035 samples and the efforts and time of the

laboratory staff which was considered as part of their daily work.

However, we could not find much relevant literature data on proficiency testing studies concerning TTI screening of blood donors.

5. CONCLUSION

The participation in an EQA programme such as B-PTS study has great impact on the quality and safety because it provides an objective and independent evaluation of the overall performance of the laboratory. Managing the non-satisfactory PTS results is a complex analytical process which should be documented and performed in a controlled manner which demands lots of experience, honesty and courage. Appropriate corrective and preventive measures should be taken in order non-conformities not to repeat. To avoid possible errors, the laboratory personnel should receive adequate and continuous training. We hope to participate in B-PTS studies on regular basis in future in order to improve the performance of our TTI testing laboratories which is one of the cornerstones of blood safety.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The program management data are no confidential. The second author of this study is the Quality Manager of the Institution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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