Short Research article

External quality assessment of transfusion-transmissible infections testing

ABSTRACT

Aims: To assess the overall performance of the transfusion-transmisible 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) on behalf of the Council of Europe's European Committee on Blood Transfusion. Participants were requested to test samples of the panel in their established, routinely used assay and report results on the online result data sheet, together with the name of the assay used.

Place and Duration of Study: Institute of blood transfusion of Macedonia, Blood testing laboratories in Skopje, Bitola and Stip, in July 2017.

Methodology: We received three sets of PTS-samples each containing 4 panels which were distributed to our laboratories. Anti-HCV panel (B-PTS032) was composed of 5 samples, anti-HIV/p24 panel (B-PTS033) of 6 samples, anti-Treponema panel (B-PTS034) of 4 samples and HBsAg panel (B-PTS035) was composed of 7 samples. The samples were subjected to serological testing with two assays: enzyme immunoassay (EIA) with Enzygnost system, Siemens using BEP2000 and chemiluminescent micro particle immuno assay (CMIA) with Architect system, Abbott using Architect i2000.

Results: According to the EDQM reports the laboratories were classified as "satisfactory" for B-PTS032: anti-HCV and B-PTS034: anti-Treponema. For B-PTS033: HIV/p24 the classification was "non evaluable" because the results for sample 6 were not properly submitted and were not included in the report. Concerning B-PTS035: HBsAg testing, laboratories were classified as "unsatisfactory" because two laboratories reported the reactive sample 3 as "Not Reactive" with the Enzignost assay and one laboratory reported the reactive sample 3 as "Not Reactive" with the Architect assay. The observed non-conformity was the S/Co value of the positive control for Architect HBsAgQ2 assay of 1.22 which was lower than the expected S/Co rang 1.65-4.96 for the used reagent lot.

Conclusion. The participation in a B-PTS study is of great importance 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. 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 immuno assay; NAT: Nuclear 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 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 EIA, CMIA and recently NAT testing has 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 HIV is recommended as a routine and is considered mandatory in most of the countries world-wide (2).

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 from 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 the three Macedonian TTI testing laboratories 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 root-cause analysis of the non-satisfactory 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 1.1mL 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 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 micro particle immuno assay (CMIA) with Architect system, Abbott using auto analyzer Architect i2000.

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 Table 1 and Table 2 respectively.

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** 1.24	**NR** 0.298	**R** 1.73	**R** 0.486	**R** 1.29	**NR** 0.273
2-NR/R	**R** 1.44	**R** 0.439	**R** 1.72	**R** 0.627	**R** 1.43	**R** 0.370
3-NR	**NR** 0.08	**NR** 0.022	**NR** 0.10	**NR** 0.073	**NR** 0.07	**NR** 0.014
4-R	**R** 3.76	**R** 0.957	**R** 5.80	**R** 1.274	**R** 4.49	**R** 0.817
5-R	**R** 4.39	**R** 1.093	**R** 6.07	**R** 1.432	**R** 4.64	**R** 0.980

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

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

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** 16.69	**R** 0.067	**R** 16.06	**R** 0.090	**R** 18.60	**R** 0.050
2-NR	**NR** 0.05	**NR** 1.992	**NR** 0.05	**NR** 1.862	**NR** 0.04	**NR** 1.920
3-R	**R** 6.73	**R** 0.583	**R** 7.05	**R** 0.498	**R** 6.95	**R** 0.513
4-R	**R** 4.41	**R** 0.446	**R** 4.44	**R** 0.480	**R** 4.78	**R** 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).

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)

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

Concerning B-PTS035: HBsAg testing, laboratories 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.

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)

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 ITM. 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, 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 milion donation 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 the participation in an external quality assurance programme. First of all we realized that some corrective and preventive measures are needed because of the unsatisfactory results in the PTS study. 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).

For that purpose we established procedure for the management of non-satisfactory PTS results which started with the documentation of the NC and of the investigation plan which was approved by the quality manager (QM). After that root-cause analysis (RCA) was performed. We investigated possible causative factors which might influence the quality of laboratory performance during the preanalitical (handling and storage of the samples), analytical (malfunction of the instrument, etc.) and post analytical phase (misreading or misinterpretation of data).

We checked environmental factors (room temperature), storage conditions of reagents (integrity and expiry date), instrument maintenance and calibration, validation of the assay, as well as verification of data transmission and interpretation process.

We also looked back at the laboratory documentation at the time of B-PTS samples testing concerning data of the environmental conditions, temperature of the refrigerators, reagent lot and the results of the run controls which were used.

We noticed that the S/Co value of the positive control for Architect HBsAgQ2 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).

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. Therefore, we performed two repeated testing of the B-PTS035 panel, but with the different lot of reagent and controls. 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.

Our analysis of the possible causative factors for the non-satisfactory PTS results indicated non-conformant performance in the analytical phase for both assays 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).

Thus, we observed that the validation criteria for the Architect HBsAg Qualitative II assay were not interpreted correctly by the laboratory. 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.

As a corrective measure we organized additional staff training and education. As a preventive measure we informed the manufactures and ask them for additional check of the instruments, as well as the validation and calibration criteria.

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

5. CONCLUSION

The participation in a 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Global Blood Safety and Availability: Facts and Figures from the 2008 Blood Safety Survey. WHO Fact Sheet No 279, June 2011.
2. European directorate for quality of medicines and healthcare (EDQM)/Council of Europe. Guide to the preparation, use and quality assurance of blood products. 19th ed. 2017.

3. Directive 2001/83/EC of the European Parliament and of the Council of 27 January 2003 on ssetting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components. (OJ L 33, 8.2.2003, p. 30).
4. European directorate for quality of medicines and healthcare (EDQM)/Council of Europe. Guidance for root-cause analysis of non-satisfactory external quality assessment results. 1st ed. 2017. https://go.edqm.eu/BPTS
5. K Soldan, K Davison, B Dow. Estimates of the frequency of HBV, HCV and HIV infectious donations entering the blood supply in the United Kingdom, 1996 to 2003. Eurosurveillance, Volume 10, Issue 2, 01 February 2005.
6. Susan L. Stramer, Ulrike Wend, Daniel Candotti, et all. Nucleic Acid Testing to Detect HBV Infection in Blood Donors N Engl J Med 2011;364:236-47.
7. PHB Bolton-Maggs (Ed) D Poles et al. on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2016 Annual SHOT Report (2017). www.shot.org
8. Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in Laboratory Medicine. Clinical Chemistry 2002; 48(5): 691-698.