Original papers

Preanalytical external quality assessment of the Croatian Society of Medical Biochemistry and Laboratory Medicine and CROQALM: finding undetected weak spots

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Abstract

Introduction: The aim of this paper is to present results of first two years of preanalytical external quality assessment (EQA) in Croatia.

Materials and methods: This paper summarizes results from 6 rounds of preanalytical EQA during 2014-2016 in 161-175 Croatian laboratories (number ranged between cycles). EQA was designed as an online survey of the compliance with National recommendations for phlebotomy (NRP). Forty-seven questions in 5 categories are analyzed (materials and equipment, patient identification, patient preparation, sampling and storage). Additionally, preanalytical cases are presented. Overall performance scores (Question score (Qscore) for compliance with NRP and Case score (Cscore) for preanalytical cases) are calculated for each question/case as a proportion of laboratories with satisfactory procedure (x 100). Qscores and Cscores ≥ 70 were classified as acceptable (maximal score = 100).

Results: In investigation of compliance with NRP, acceptable Qscores were obtained for 34/47 questions. The lowest scores were observed for the availability of sterile disposable tourniquets (Qscore = 15) and safe-sharp needles (Qscore = 34), obtaining patients address as an identifier (Qscore = 21), using glycolysis inhibitor tubes for glucose concentration measurement (Qscore = 21) and verification of manufacturers declarations on temperature and time of storage (Qscore = 31). There was no statistically significant difference in overall Qscore according to different categories of phlebotomy procedures (P = 0.284). The results of preanalytical cases showed acceptable Cscore values for all cases (89-96).

Conclusion: First two years of preanalytical EQA showed good compliance with the NRP and excellent expertise in resolving complex preanalytical issues. Major critical spots are lack of availability of safe-sharp needles, disposable tourniquets and glucose inhibitor tubes.

Key words: preanalytical phase; external quality assessment; laboratory error

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Introduction

External quality assessment (EQA) of the analytical phase of laboratory work has, for some time, been established as an essential tool for quality assurance. Many different providers available at the market ensure that laboratories can choose the schemes that best meet their needs regarding the cost, sample quality, time of delivery or quality of reports. The ISO 15189:2012 states that "External quality assessment programs should, as far as possible, provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre- and post-examination procedures" (1). Laboratories should, therefore, additionally participate in EQAs that cover extra-analytical phase of laboratory work.

According to Kristensen GB *et al.*, there are three main types of preanalytical EQA (2). Type I refers to registration of procedures. This type includes questionnaires where participants are asked to declare their practices regarding certain aspects of preanalytical phase. Even though the most simple to organize, Type I preanalytical EQA still provides valuable information on management of preanalytical phase in laboratories (2). In Type II, samples with preanalytical errors (e.g. hemolysis, lipemia, icterus) are sent to laboratories for processing. This type of EQA can be costly and production of adequate materials can be analytically challenging. In Type III EQA, preanalytical errors or quality indicators are continuously monitored and recorded into databases (3,4). However, in order to obtain valuable information from this data, participating laboratories need to regularly record preanalytical errors during their routine work. Also, definition of particular preanalytical errors can differ between laboratories.

The first efforts in recording the state of management of extra-analytical phase in Croatia was done in 2009 (5). This study discovered unacceptably low awareness of the importance of preanalytical phase, especially regarding phlebotomy procedures. Similar results were conformed on a wider level in a subsequent multicentre investigation of the quality of the extra-analytical phase of laboratory practice in some developing European countries and Mexico (6). These alarming results motivated Croatian Society of Medical Biochemistry and Laboratory Medicine to form a Working group for the preanalytical phase (WGPA CSMBLM) in 2012, and implement preanalytical External Quality Assessment in Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) in 2014. Up to now, only Type I preanalytical EQA has been conducted. As a first step in improving phlebotomy procedures, National recommendations for venous blood sampling were issued (7). The main goal of preanalytical EQA was to get the overview of the quality of policies and procedures in Croatian laboratories, identify room for improvement and encourage harmonization of preanalytical practices.

The aim of this paper is, by presenting data collected by two-year preanalytical EQA, to assess the i) level of compliance to the National recommendations for phlebotomy (NRP) (7); ii) level of expertise in resolving complex preanalytical issues presented in preanalytical cases. Moreover, we aimed to identify, otherwise overlooked, most critical steps in the preanalytical phase on a national level.

Materials and methods

CROQALM is the Croatian External quality provider offering different analytical modules for various fields of laboratory diagnostics (general and specific clinical chemistry, haematology and coagulation, cardiac markers, urinalysis, blood gas testing, immunochemistry and HbA1c). Each module has appointed module coordinator, who is responsible for the general strategy of the specific EQA, including data analysis and interpretation. In September 2014, CROQALM introduced two new extra-analytical modules in the EQA scheme: preanalytical and postanalytical module. During the 2014, only one cycle of the preanalytical EQA was conducted. This cycle included 3 preanalytical cases from the routine practice. From the year 2015, preanalytical scheme is conducted three times a year. All three cycles in the 2015 and the first cycle in the 2016 included questionnaire about the compliance with the National recommendations for venous blood sampling (7). The second cycle in the 2016 again included 3 preanalytical cases. This paper summarizes results of these first six cycles up to June 2016.

Invitation for participation in the preanalytical EQA module is sent out to all Croatian laboratories. Total number of invited laboratories varied slightly across the time (in 2014: N = 180; for three cycles in 2015: N = 194, 192 and 191, respectively; for two cycles in 2016: N = 198).

Participation in the scheme is free of charge for all Croatian laboratories. For other analytical EQA modules in CROQALM, acceptance criterion for each parameter is set and based on the laboratory performance; percentage of agreement is calculated by the module coordinator. For the preanalytical module, answers of the participants are not

scored. Answers that differed from the expected answers were not recorded as errors. Data analyses and explanations are provided for educational purposes only.

Preanalytical modules are conducted using web service and software for CROQALM (*inlab2*QALM*, In2 group, Croatia). Head of each laboratory (or person responsible for the EQA within laboratory) has unique username and password for accessing questions and entering results. Opening of the cycle is announced *via* e-mail notification. Upon initiation of the cycle, cases or questions are available in the web based software *inlab2*QALM*. Within 15 days upon opening, laboratory heads are required to submit answers which reflect procedures conducted in their laboratories. After completion of the cycle, participants' answers and data on participating laboratory types are exported from the database and analysed by the preanalytical module coordinator.

Questionnaire

Four cycles of the preanalytical EQA were designed to investigate compliance with procedures described in the National recommendations for venous blood sampling (7). Each cycle covered one area of the phlebotomy procedure: a) materials and equipment, b) patient identification, c) patient preparation, d) sampling and e) storage (patient preparation and sampling were addressed in the same cycle). Total of 47 questions is included. Participants were asked to report if the procedures were implemented in their laboratories by closed questions with one of following answers: yes, no or sometimes. All questions were formulated in a way that answer "yes" is a desirable answer. "Non applicable" option was also available.

Preanalytical cases were distributed across two cycles. Four possible answers were available for each case. Participants were asked to choose answer which best describes their laboratory procedure in the presented case.

Statistical analysis

Individual data are presented as counts and percentages.

In order to compare overall participants performance on specific questions in the part of the questionnaire that investigated compliance with the recommendations, Question score (Qscore) was created. Numerical value is attributed to each possible answer: "yes" – 2 points, "sometimes" – 1 point and "no" – 0 points. Answers "non applicable" were excluded from the analysis. Since there was unequal number of participants among cycles, a maximum score was calculated for each question (as if all participants answered "yes"; number of participants x 2). Then, actual participants' answers were summarized according to formula:

Participants score = (number of "yes" answers x 2) + (number of "sometimes" answers x 1).

Qscore was presented as a whole number and calculated according to formula:

Qscore = (participants score / maximum score) x 100.

For the comparison of participants performance in preanalytical case studies, Case score (Cscore) was calculated. Numerical value is attributed to each possible answer: from 0 to 5 points. Numerical values were assessed by the module coordinator. 5 points was attributed to the most desirable procedure regarding the case, 0 points was attributed to the most erroneous procedure; points in between were attributed to acceptable procedures based on the level of deviation from the most desirable procedure. Since there was unequal number of participants among cycles, a maximum score was calculated for each case study (as if all participants chose the most desirable procedure, number of participants x 5). Then, actual participants' answers were summarized according to formula:

Participants score = (number of "5 point" answers x 5) + (number of "4 point" answers x 4) + *etc.*

Cscore was presented as a whole number and calculated according to formula:

Cscore = (participants score / maximum score) x 100.

Qscores and Cscores ≥ 70 were classified as acceptable.

Difference in overall Qscore according to different categories of phlebotomy procedures: 1) materials and equipment, 2) patient identification, 3) patient preparation and sampling (these categories were combined due to the small number of questions in the patient preparation category) and 4) transportation and storage was tested using Kruskal Wallis test. P values < 0.05 were considered statistically significant.

Statistical analysis was done using Microsoft Excel (version 2010, Microsoft, USA) and MedCalc (MedCalc Software, version 11.5.1.0, Ostend, Belgium).

Results

Number of participating laboratories, response rate and distribution of types of laboratory are presented in Table 1. Response rate was high and ranged from 84.8% to 90.2%.

Table 2 presents summary of results to the self-reporting part of the questionnaire regarding compliance with procedures described in the National recommendations for venous blood sampling (7).

Overall, acceptable Qscores were obtained for 34/47 items included into questionnaire investigating compliance with the venous blood sampling procedures described in the National recommendations. However, there were some procedures with unexpectedly low compliance. The lowest scores were observed for the availability of sterile disposable tourniquets (#9, Qscore = 15) and safe-sharp needles (#4, Qscore = 34) in the category equipment, obtaining patients address as an identifier in category identification (#15, Qscore = 21), using exclusively tubes with glycolysis inhibitor for glucose concentration measurement (#40, Qscore = 21) and verification of manufacturers declarations on temperature and time of storage (#47, Qscore = 31) in the category transport and storage. Almost complete compliance (Qscores > 98) was recorded for repeating unsuccessful phlebotomy at different puncture site (#30), following manufacturer's declarations on temperature and time of storage of samples (#46), measuring coagulation tests within 4 hours from sampling (#44), monitoring allowed time from sampling to measurement for urinalysis (#43) and marking tubes with laboratory ID number (#19).

There was no statistically significant difference in overall Qscore according to different categories of phlebotomy procedures (P = 0.284) (Figure 1).

Table 1. Number of participating laboratories, response rate and distribution of laboratory types.

	2014 Cycle, N (%)	2015 Cycle 1, N (%)	2015 Cycle 2, N (%)	2015 Cycle 3, N (%)	2016 Cycle 1, N (%)	2016 Cycle 2, N (%)
Primary healthcare laboratories	81 (50.3%)	90 (51.4%)	85 (50.3%)	85 (50.6%)	85 (50.0%)	85 (50.6%)
Hospital laboratories	80 (49.7%)	85 (48.6%)	84 (49.7%)	83 (49.4%)	85 (50.0%)	83 83 (49.4%)
General hospital	17 (10.6%)	18 (10.3%)	17 (10.1%)	17 (10.1%)	18 (10.6%)	17 (10.1%)
County hospital	6 (3.7%)	6 (3.4%)	6 (3.6%)	5 (3.0%)	6 (3.5%)	6 (3.6%)
University and specialized hospitals	20 (12.4%)	22 (12.6%)	22 (13.0%)	23 (13.7%)	23 (13.5%)	23 (13.7%)
University hospital centres	10 (6.2%)	11 (6.3%)	11 (6.5%)	10 (6.0%)	10 (5.9%)	9 (5.4%)
Specialized polyclinic	3 (1.9%)	4 (2.3%)	4 (2.4%)	4 (2.4%)	3 (1.8%)	4 (2.4%)
Private polyclinic	22 (13.7%)	24 (13.7%)	24 (14.2%)	24 (14.3%)	25 (14.7%)	24 (14.3%)
Total number of participating laboratories	161	175	169	168	170	168
Total number of invited laboratories	180	194	192	191	198	198
Response rate	89.4%	90.2%	88.0%	88.0%	85.8%	84.8%

Table 2. Self-reporting answers regarding compliance to the National recommendations for venous blood sampling in CROQALM EQA.

#	Question	Y (N)	N (N)	S (N)	Qscore
Materials and equipment					
1	Written instruction for blood sampling is available at the phlebotomy station.	163	10	1	94
2	Different diameter needles are available at the phlebotomy station.	153	14	7	90
3	Butterfly needles are available at the phlebotomy station.	120	36	17	74
4	Safe-sharp needles are available at the phlebotomy station.	48	105	20	34
5	Different volume tubes are available at the phlebotomy station.	132	29	13	80
6	Sterile pads for disinfection of the puncture site are available at the phlebotomy station.	94	71	8	57
7	Sterile disposable gloves are available at the phlebotomy station.	134	34	3	79
8	Sterile disposable holders are available at the phlebotomy station.	75	86	11	47
9	Sterile disposable tourniquets are available at the phlebotomy station.	22	144	7	15
10	Professional phlebotomy chair is available at the phlebotomy station.	79	2	91	72
11	Disposable containers are available at the phlebotomy station.	154	13	5	91
Patient identification					
12	Patient has to provide identification document prior to blood sampling.	71	64	34	52
13	Prior to blood sampling, phlebotomist asks the patient to identify himself.	159	3	6	96
14	Prior to blood sampling, phlebotomist asks the patient about the date of birth.	93	26	49	70
15	Prior to blood sampling, phlebotomist asks the patient about the address.	13	109	45	21
16	All tubes are labeled with barcode.	98	66	1	60
17	Patient's name is present on each tube.	148	13	8	90
18	Patient's date of birth is present on each tube.	68	60	39	52
19	Laboratory ID number is present on each tube.	164	4	0	98
20	At least two identifiers are present on each tube.	141	20	7	86
21	Tubes are labeled prior to blood sampling.	152	13	4	91
22	The name of phlebotomist is recorded for every tube.	108	42	18	70
23	Time of sampling is recorded for every tube.	125	25	17	80
Patient preparation					
24	Fasting status is checked prior to sampling.	147	5	15	93
25	If the patient is not fasting, phlebotomy is postponed.	149	3	15	94
Sampling					
26	Tourniquet is always used during the sampling.	130	32	6	79
27	Phlebotomist puts on gloves before applying tourniquet.	144	8	16	90
28	In order to avoid hemolysis and burning sensation, phlebotomist waits for disinfectant to evaporate before the puncture.	145	19	4	88
29	Evacuated tubes are not used when Sampling has to be repeated due to problems during phlebotomy (insufficient blood flow).	52	60	56	48
30	Sampling is repeated at different puncture site when the first phlebotomy was unsuccessful.	166	0	2	99
31	Tourniquet is released when blood starts to flow into first tube.	142	11	15	89
32	Tubes are mixed immediately after phlebotomy, before the needle is removed from the vein.	133	29	6	81
33	Coagulation tube is drawn before the serum tube.	142	20	5	87

#	Question	Y (N)	N (N)	S (N)	Qscore
Transport and storage					
34	Laboratory monitors time from phlebotomy to sample admission for tubes sampled outside of laboratory.	118	27	/	81
35	Laboratory monitors temperature conditions for tubes sampled outside of laboratory.	102	36	/	74
36	Laboratory has written procedures for delivering samples by postal services.	39	34	/	53
37	Laboratory has criteria for rejecting samples due to nonconformities during delivery.	112	35	/	76
38	Only samples delivered on ice are accepted for ammonia concentration measurement.	34	3	/	92
39	Only samples delivered on ice are accepted for lactate concentration measurement.	26	9	/	74
40	Only tubes with glycolysis inhibitor are accepted for glucose concentration measurement.	30	112	/	21
41	For adrenocorticotropic hormone (ACTH) measurement, blood is sampled in cooled tubes and immediately placed on ice.	24	7	/	77
42	Laboratory declares maximal allowed time from sampling to analysis for blood gas testing and rejects samples that are not fulfilling criteria.	43	7	/	86
43	Laboratory declares maximal allowed time from sampling to analysis for urinalysis and rejects samples that are not fulfilling criteria.	157	4	/	98
44	All coagulation tests are done within 4 hours from sampling.	152	3	/	98
45	Laboratory collects and stores sampled for the longer period of time for certain tests (tests are not done immediately).	70	96	/	42
46	Laboratory follows manufacturer's declarations on temperature and time of storage.	151	2	/	99
47	Laboratory has verified manufacturer's declarations on temperature and time of storage.	46	102	/	31

Y – yes; N – no; S – sometimes; / – answer not provided in the questionnaire.

Results of the 6 preanalytical cases are presented in Tables 3 to 8. In all cases, calculated Cscores acceptable and ranged from 89 to 96.

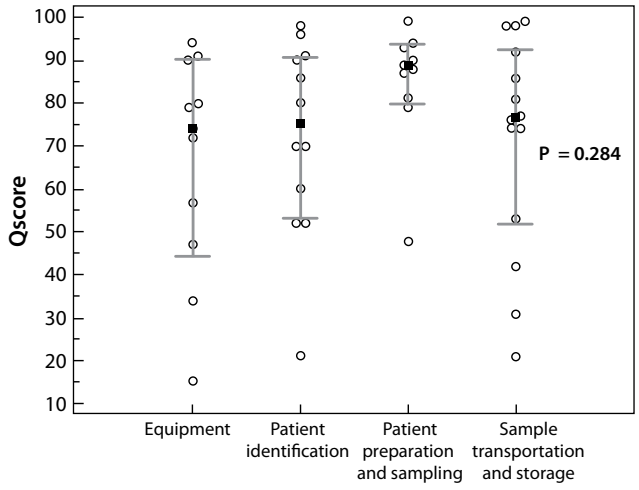

Figure 1. Qscore according to different categories of phlebotomy procedure.
Individual data are presented with white circles, median value is presented with black square, 95% confidence interval of the median is presented with grey line.

Case 1 was presented as follows: Patient arrives to laboratory with 24-hour urine for creatinine clearance testing. Urine is collected into clean plastic bottle (volume 1.5 litres) and the bottle is completely filled. After confirming collection technique with the laboratory staff, patient admits that one small part of the first morning urine on the second day (by patient's estimation 1 to 2 dL) was discarded because the collection bottle was already completely filled. Please select answer which best describes laboratory procedure in the presented case.

Results, available answers and overall case score are presented in Table 3. Overall case score was 94. Neither laboratory chose the least desirable procedure.

Case 2 was presented as follows: Patient arrives to laboratory at 11 am with a request for the oral glucose tolerance test (oGTT). The patient is still fasting and has not consumed any food and

Table 3. Preanalytical cases in CROQALM EQA: results, available answers and overall case score for Case 1.

Case 1	N = 151	Points	Max case points	Case points	Cscore
1. the sample is accepted for testing, the result is reported without any comments	0	0	755	713	**94**
2. although the sample is inadequate it is accepted for testing, but the result is reported with an interpretative comment	9	3			
3. the sample is inadequate and is not accepted for testing	136	5			
4. although the sample is inadequate, in exceptional situations may be accepted if the patient or ordering physician explicitly require it	6	1			

drink. Please select answer which best describes laboratory procedure in the presented case.

Results, available answers and overall case score are presented in Table 4. The lowest overall case score was recorded for this case (Cscore = 89). 4 out of 160 laboratories chose the least desirable procedure.

Case 3 was presented as follows: The laboratory has received the serum sample with a request for potassium measurement. The sample is slightly haemolysed (free haemoglobin concentration of 0.5 g/L; Figure 2: tube No. 3). Please select answer which best describes laboratory procedure in the presented case.

Results, available answers and overall case score are presented in Table 5. Overall case score was 92. Neither laboratory chose the least desirable procedure.

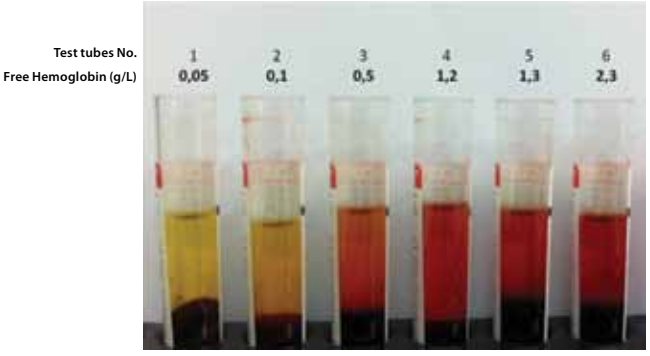

Figure 2. Coloured scale accompanying the preanalytical Case 3.

Case 4 was presented as follows: An outpatient sample for prothrombin time (PT) measurement is delivered to laboratory. The tube was sampled in the patient's home by medical staff and delivered to laboratory by courier service. The service is not covered by insurance; patient is paying for his laboratory testing. The citrate tube is not completely filled; plasma level is 0.5

Table 4. Preanalytical cases in CROQALM EQA: results, available answers and overall case score for Case 2.

Case 2	N = 160	Points	Max case points	Case points	Cscore
1. the sample is accepted for testing, the result is reported without any comments	4	0	800	712	**89**
2. although the sample is inadequate it is accepted for testing, but the result is reported with an interpretative comment	10	3			
3. the sample is inadequate and is not accepted for testing	134	5			
4. although the sample is inadequate, in exceptional situations may be accepted if the patient or ordering physician explicitly require it	12	1			

Table 5. Preanalytical cases in CROQALM EQA: results, available answers and overall case score for Case 3.

Case 3	N = 159	Points	Max case points	Case points	Cscore
1. the sample is accepted for testing, the result is reported without any comments	0	0	795	733	**92**
2. although the sample is inadequate it is accepted for testing, but the result is reported with an interpretative comment	15	3			
3. the sample is inadequate and is not accepted for testing	136	5			
4. although the sample is inadequate, in exceptional situations may be accepted if the patient or ordering physician explicitly require it	8	1			

cm below the lower acceptable mark. Please select answer which best describes laboratory procedure in the presented case.

Results, available answers and overall case scores are presented in Table 6. Overall case score was 92. 3 out of 168 laboratories chose the least desirable procedure.

Case 5 was presented as follows: Laboratory receives samples from the general physician offices in the area. Due to the problems with the delivery, a sample drawn at 8 am is delivered to laboratory at 1 pm. Blood is collected into serum tube with clot activator without gel. C-reactive protein (CRP) and glucose are ordered. Please select answer which best describes laboratory procedure in the presented case.

Results, available answers and overall case score are presented in Table 7. The lowest overall case score, the same as in Case 2 was recorded for this case (Cscore = 89). 1 out of 165 laboratories chose the least desirable procedure.

Case 6 was presented as follows: At 10 am biochemistry measurement is performed in the serum sample with gel separator in the emergency laboratory. Upon measurement, samples are stored at room temperature without stoppers. Since the patient's clinical symptoms are still unclear, ordering physician requests ethanol measurement at 3 pm by phone. Please select response which best describes laboratory procedure with this sample.

Results, available answers and overall case score are presented in Table 8. The highest overall case score was recorded for this case (Cscore = 96). Neither laboratory chose the least desirable procedure.

Table 6. Preanalytical cases in CROQALM EQA: results, available answers and overall case score for Case 4.

Case 4	N = 168	Points	Max case points	Case points	Cscore
1. the sample is inadequate and is not accepted for testing	142	5	840	769	**92**
2. the sample is accepted for testing, PT result is reported with an accompanying comment on insufficient plasma volume	18	3			
3. the sample is accepted for testing, PT result is reported without any accompanying comments	3	0			
4. although the sample is inadequate, since it was delivered from patients home, in this exceptional situation sample will be accepted for PT testing (hospital samples with insufficient volume are rejected)	5	1			

Table 7. Preanalytical cases in CROQALM EQA: results, available answers and overall case score for Case 5.

Case 5	N = 165	Points	Max case points	Case points	Cscore
1. CRP and glucose will be measured and reported in this sample without any comments	1	0	825	734	**89**
2. CRP and glucose will be measured and reported from the serum sample, an interpretative comment on influence of sampling time on glucose concentration will be added	24	3			
3. The result for CRP will be reported, the result for glucose will be suppressed	102	5			
4. The sample is inadequate, tests will not be measured in this sample	38	4			

Table 8. Preanalytical cases in CROQALM EQA: results, available answers and overall case score for Case 6.

Case 6	N = 147	Points	Max case points	Case points	Cscore
1. The sample is unacceptable, the measurement of ethanol will not be done	135	5	735	708	**96**
2. Ethanol will be measured and result reported without any comments.	0	0			
3. Ethanol will be measured, but the result will be reported without an interpretative comment on possible falsely decreased ethanol concentration	11	3			
4. Ethanol will be measured only if the electronic or paper request is delivered to laboratory	1	0			

Discussion

Overall, the first 2-year experience of preanalytical EQA of the Croatian Society of Medical Biochemistry and Laboratory Medicine and CROQALM show good compliance with recommended phlebotomy procedures and good management in challenging preanalytical case studies. However, these results identify some unexpected weak spots in the preanalytical phase that could have detrimental effect on results in some laboratories.

In 2013, WGPA CSMBLM recognized a need for implementing nationwide standard in phlebotomy and issued a document Croatian Society of Medical Biochemistry and Laboratory Medicine: national recommendations for venous blood sampling (7). Document was distributed to all members of the CSMBLM society free of charge and several educational events were organized to assure implementation of the procedure. In 2014, EQA in preanalytical phase aimed to investigate compliance with the recommended procedure.

Several items scored very low in the section dealing with materials and equipment. Sterile disposable tourniquets were present only in small proportion of laboratories. In Croatia, most laboratories use reusable rubber or fabric tourniquets. Recently, a microbiological investigation of reusable tourniquets was conducted in University Hospital Center Sestre Milosrdnice (Zagreb, Croatia) (8). The results of this study show average tourniquet duration of 165 (90–360) days. Only 17% of participants wash tourniquet daily and only 27% disinfect tourniquet daily. Microbiological analysis showed that some bacterial flora was detected on all but two tourniquets. *Bacillus sp*. were detected on 5/52, *Enterococcus sp*. on 2/52, *Methicillin-resistant Staphylococcus aureus* (MRSA) on 2/52 and *S*.

aureus on 1 out of 52 tested tourniquets. These results are in agreement with several other previously published studies (9,10). Elhassan HA and Dixon T have identified 18/50 tourniquets positive for *S. aureus* and 6/50 positive for MRSA (11). These results show that reusable tourniquets pose serious health risk, not only for patients, but also for the medical professionals. Laboratories are encouraged to use disposable tourniquets.

The other important issue arisen from this section is low distribution of safe-sharp needles. Although being in effect for several years, The Directive 2010/32/EU "Prevention from sharp injuries in the hospital and healthcare sector" (12) has not been implemented in Croatian laboratories. There is an ample of evidence that safe-sharp laboratory materials significantly lower risk of needle-stick injuries (13). Even though the main reason for delaying implementation of safe-sharp equipment is increased cost for the laboratory, the overall cost for the diagnostics and treatment of needle-stick injuries for the healthcare in general is higher (14,15).

Results of EQA detected no critical areas in the section dealing with patient identification. Isolated low Qscore for obtaining patients address only points to the non-standardized types of questions used for patient identification. The most reliable way of providing identification document is not widespread in Croatia. There is also a great variability in tube labelling. While almost perfect score (Qscore = 98) was obtained for the patient ID on the tube, other identifiers (patients name and patients date of birth) were present with the lower frequency. Nevertheless, Qscore for two identifiers present at each tube was rather high (Qscore = 86). Patient identification is the most crucial step in the phlebotomy process. In the recently published survey by the Working group for the preanalytical phase of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM WG-PRE), patient identification and test tube labelling were identified as the key guideline issues with the highest combination of occurrence probability and potential risk of harm (16). Recognizing the importance of this issue, EFLM WG-PRE issues a call for harmonization in patient identification and tube labelling (17). Since harmonization of this phase of the process is not yet achieved in Croatian laboratories, overall good performance can certainly be improved by implementation of EFLM WG-PRE standards.

Verification of patient preparation scored extremely well in the Croatian preanalytical EQA. However, only fasting status was presented in a preanalytical case and no other specific preparations were investigated here. Working Group for Patient Preparation of the Croatian Society of Medical Biochemistry and Laboratory Medicine (WGPP CSMBLM) recently published results of a survey on instructions for patient preparation in Croatian laboratories (18). Although results showed that instructions are not standardized and that the information is lacking for some specific areas like therapeutic drug monitoring, coagulation and endocrinology, instructions in the field of clinical chemistry were rather uniform. This is probably a result of availability of patient preparation instructions in the field of clinical chemistry (19). The study discovered that adherence to existing guidelines is rather high, but where guidelines are lacking, laboratories fail to issue their own guidelines (18). WGPP CSMBLM identified the need for the standardized evidence based guidelines for all areas of laboratory medicine. Adherence to the EFLM WG-PRE recommendation that patients should be fasting prior to phlebotomy and that phlebotomy should be postponed if the patients are not fasting was very high (20,21).

Phlebotomy technique seems to completely be adherent to the published national standards for venous blood sampling. All steps scored extremely high. The only area where potential for improvement was detected was the repeated phlebotomy. Hence, when repeating unsuccessful phlebotomy, most laboratories use evacuated tubes, same as the initial phlebotomy. There is evidence that tubes using manual aspiration are more suitable in these situations because lower force reduces stress and the consequent injury of blood cells in comparison with the evacuated tubes and lowers the risk of hemolysis and prevents hyper-activation of platelets (22, 23). These tubes could, therefore, be better for repeated blood sampling.

Transportation and storage is also extremely important preanalytical requirement (24). Unfortunately, some items scored very low in this area. An issue of glucose tube arose from this questionnaire. Very low score (QScore = 21) was recorded for exclusive usage of glycolysis inhibitor tubes for the glucose concentration measurement. It is well documented that glucose concentration decreases over time in serum and plasma tubes without the inhibitor. Juricic *et al*. recently published an article comparing glucose concentration in four different types of tube: serum without additive, lithium heparin plasma, sodium flouride/potassium oxalate (NaF/KOx) plasma and liquid citrate plasma. They have found that if all tubes were centrifuged within 30 minutes and measurement done immediately, there is a statistically significant difference in glucose concentration. The highest drop of glucose concentration was observed in the serum tube, then in NaF/KOx followed by lithium-heparin tube sampled on ice. Liquid citrate tube preserved glucose concentration the best (25). These results underline the risks associated with usage of serum tube for glucose concentration measurement: misdiagnosis of diabetes patients and falsely decreased glucose concentration. Even more relevant were the results of another study by the same group of authors where the stability of glucose concentration in uncentrifuged tubes was investigated. These results clearly show that only the tube with liquid citrate can preserve glucose concentration in uncentrifuged tubes for the period of three hours (26). In Croatian primary care laboratories, sampling is done on phlebotomy sites and general physician offices, samples collected and transported to larger hospital centres for processing (27). Since these areas are sometimes more than 100 km apart, several hours may pass from the sample collection to the glucose concentration measurement. In these cases, it is necessary to introduce glycolysis inhibitor tube always when glucose concentration is ordered.

Many laboratories collect and store samples for the analyses that are rarely done. Samples are than analysed in batch in order to cut costs on calibration and control materials without compromising quality of results and patient safety. Almost all laboratories declared (Qscore = 99) that they follow manufacturers declarations regarding temperature and time of storage. However, only small proportion (Qscore = 31) has declared that they have verified those conditions. Manufacturers declarations are often not confirmed in the routine laboratory work (28,29). Recently we have published results of verification of storage conditions for angiotensin-converting enzyme (ACE kinetic, Bühlman Laboratories AG, Schonenbuch, Switzerland). Although the manufacturer has declared that the analyte is stable up to 6 months when frozen on -20 ^{0}C, we have discovered that the decline in enzyme activity is unacceptable after 1 week (30). These results underline the need of each laboratory to verify manufacturer's declarations on analyte stability if samples are going to be stored prior to measurement.

Overall laboratory performance was very high in the preanalytical cases section, which confirms that laboratories are well educated and equipped to resolve complex preanalytical issues. The lowest score was observed in the case dealing with sample stability for the glucose concentration measurement. While certain number of laboratories saw nothing unacceptable in reporting the result for glucose concentration from the sample that has been drawn 5 hours before the admission to laboratory, another proportion turned down reporting result for the stable analyte (CRP). The intention of laboratory should always be to report as many results as possible while maintaining quality of the measured result. If some parameters can't be measured from the sample, sample should be partially analyzed in order to spare the patient of repeated sampling for all tests (smaller volume tubes can be sampled when sampling needs to be repeated).

Performance in the case dealing with haemolysed sample was rather high. Largest proportion of laboratories does not report potassium concentration from the haemolysed sample, while smaller number report values with an interpretative comments. A good knowledge of the proper detection and management of haemolysed, icteric and

lipemic samples has been documented in a recently published work by the WGPA CSMBLM (31).

Our observations might have some limitations that are a direct result of the type of preanalytical EQA currently conducted in Croatia. Most of the detected problems were based on the self-reported answers provided by the laboratory heads. It is possible that they were giving desirable answers rather than describing the real situation in their laboratories. An observational study carried out as an independent laboratory audit would give a better insight into problems in the preanalytical phase. Also, even though the response rate was very high, it was not complete and there were some variations between cycles. However, small number of laboratories that failed to participate in this EQA probably would not significantly change results.

In conclusion, results of the first two years of preanalytical EQA CROQALM showed good compliance with the national recommendations for phlebotomy and excellent expertise in resolving complex preanalytical issues. Some weak spots in preanalytical phase of Croatian laboratories are identified. Safe-sharp needles and disposable tourniquets which are necessary for reduced staff and patients risk during the phlebotomy procedure are not available in all laboratories. Additionally, standardization of glucose concentration measurement regarding tube type and sample acceptance criteria should be implemented. Verification of manufacturer's procedures is necessary when samples are stored. These issues will continue to be monitored in order to achieve highest standards of laboratory work. Croatian Society of Medical Biochemistry and Laboratory Medicine and CROQALM will continue their educational efforts to improve preanalytical phase in Croatian laboratories.

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Potential conflict of interest

None declared.

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