The knowledge and understanding of preanalytical phase among biomedicine students at the University of Zagreb

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Abstract

Introduction: The educational program for health care personnel is important for reducing preanalytical errors and improving quality of laboratory test results. The aim of our study was to assess the level of knowledge on preanalytical phase in population of biomedicine students through a cross-sectional survey.

Materials and methods: A survey was sent to students on penultimate and final year of Faculty of Pharmacy and Biochemistry – study of medical biochemistry (FPB), Faculty of Veterinary Medicine (FVM) and School of Medicine (SM), University of Zagreb, Croatia, using the web tool SurveyMonkey. Survey was composed of demographics and 14 statements regarding the preanalytical phase of laboratory testing. Comparison of frequencies and proportions of correct answers was done with Fisher’s exact test and test of comparison of proportions, respectively.

Results: Study included 135 participants, median age 24 (23-40) years. Students from FPB had higher proportion of correct answers (86%) compared to students from other biomedical faculties 62%, P < 0.001. Students from FPB were more conscious of the importance of specimen mixing (P = 0.027), prevalence of preanalytical errors (P = 0.001), impact of hemolysis (P = 0.032) and lipemia interferences (P = 0.010), proper choice of anticoagulants (P = 0.001), transport conditions for ammonia sample (P < 0.001) and order of draw during blood specimen collection (P < 0.001), in comparison with students from SM and FVM.

Conclusions: Students from FPB are more conscious of the importance of preanalytical phase of testing in comparison with their colleagues from other biomedical faculties. No difference in knowledge between penultimate and final year of the same faculty was found.

Key words: survey; education; preanalytical phase

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Introduction

Laboratory diagnostics develops through different phases that span from test ordering and collection of diagnostic specimens (preanalytical phase), sample analysis (analytical phase) and results reporting and interpretation (postanalytical phase) (1). Preanalytical phase is nowadays recognized as the most vulnerable part of the total testing process. Due to their impact on the quality of results of laboratory testing, preanalytical errors have been recognized as the greatest challenges to the laboratory professionals (2). Several large surveys have been performed regarding preanalytical phase of laboratory testing (3-5). Standardization of preanalytical activities can be achieved by major adherence to available guidelines, implementation of total quality management system that include preanalytical requirements, as well as continuous education of the health care staff with test ordering and blood sampling responsibilities (6).
A correct preanalytical phase procedure is critical to get an adequate sample and consequently to achieve the most reliable laboratory results, promoting patient safety. Since the chance of errors may adversely impact the quality of testing and patient safety, the educational program for health care personnel is important for decreasing preanalytical errors and improving quality of laboratory results. In the graduate study curriculum of biomedicine in Croatia (University of Zagreb) there are different courses regarding laboratory diagnostics, obligatory or elective, depending on faculty.

The hypothesis of this study was that the level of education on preanalytical phase of laboratory work for biomedicine students from University of Zagreb is not sufficient to insure high level of quality of the tested specimens and consequently, most reliable testing results. In addition, we hypothesized that students from Faculty of Pharmacy and Biochemistry (graduate programme Master of Medical biochemistry) would have more knowledge on topics regarding preanalytical phase. The aim of our study was to assess the level of knowledge on preanalytical phase in the population of biomedicine students from University of Zagreb, Croatia, through a cross-sectional survey. Our additional aim was to assess the difference in level of knowledge between penultimate and final year of the same faculty.

**Materials and methods**

**Study design**

This study was conceived as a questionnaire on preanalytical phase, intended for the students on penultimate and final year of Faculty of Pharmacy and Biochemistry (graduate programme Master of Medical biochemistry), Faculty of Veterinary Medicine and School of Medicine, all from University of Zagreb, Croatia. An online survey composed of two parts, including demographics (student’s age, faculty and year of study) and 14 statements, was created using the web tool SurveyMonkey (Palo Alto, CA, USA). Statements were created by all authors and reflect our personal impression about the most problematic preanalytical issues.

General statements were related to sample quality, frequent preanalytical errors and sample collection, while specific statements were related to impact of hemolysis and lipemia interferences, proper choice of anticoagulants, transport conditions for ammonia sample and storage temperature for urine analysis. Selected topics represent the most frequent issues concerning laboratory and clinicians/veterinarians present in everyday practice. The survey was sent by e-mail to students with permission from faculties and with response time between June 1, 2014 and September 30, 2014. Offered answers for quoted statements were: “correct”, “incorrect” and “I do not know”.

**Participants**

Inclusion criteria for all participants were penultimate and final year of study, due to the fact that students on penultimate year are attending courses about laboratory medicine. Exclusion criteria were not belonging to the tested population of penultimate and final year students, so 8 participants of 143 students who responded to the questionnaire were excluded from the data analysis. Therefore, answers of 135 students were accepted for further statistical analysis. The questionnaire was conducted with permission from the Ethics Committee of all three faculties.

**Statistical analysis**

Data collected from the students from School of Medicine (SM) and Faculty of Veterinary Medicine (FVM) were grouped in one group and they were compared with the data collected from students from Faculty of Pharmacy and Biochemistry (FPB). Incorrect answers to the offered statements and answers “I do not know” were grouped in one category and in other category was correct answer to the offered statement. For comparison of proportions of correct answers between FPB students and SM and FVM, statistical test of comparison of proportions was used, for all statements and separately for general and specific statements. Reasons for such data grouping lie in our presumption that students from FPB will adopt more knowledge and skills from the field of preanalytics in compari-
son to their colleagues from SM and FVM. Furthermore, the practical reason for such data grouping was the inability to use chi-square test due to the low frequencies.

Fisher’s exact test was used for comparison of frequencies of correct answers between FPB and SM & FVM groups for each statement, and also for the comparison of frequencies of correct answers between penultimate and final year within each faculty. The P-value \( \leq 0.05 \) was considered statistically significant. All statistical analysis of qualitative results was done with MedCalc software, version 10.20.0 (Mariakerke, Belgium).

**Results**

The total number of students who participated in survey was 143. Eight participants were excluded from the data analysis because they did not belong to the tested population of penultimate and final year students. These students had access to online survey because they were attending some courses with penultimate and final year students, but were excluded as they were not regular students of these years of study and therefore might not be included in courses relevant for the survey. For total number of tested participants (\( N = 135 \)) median age was 24 years, range from 23 to 40 years. Response rate for participants on penultimate and final year from Faculty of Pharmacy and Biochemistry (FPB, graduate programme Master of Medical biochemistry) was 0.82 (28 / 34), for students from Faculty of Veterinary Medicine (FVM) it was 0.43 (53 / 124), and 0.10 (54 / 547) for students from School of Medicine (SM). Number of survey participants and response rate are shown in Table 1.

Results of comparison of proportions of correct answers for all statements, as well as for general and specific statements, between FPB students and SM and FVM students are shown in Table 2. Students from FPB had higher proportion of correct answers (86%) compared to students from other biomedical faculties 62%, \( P < 0.001 \). For general statements students from FPB had 91% of correct answers and 69% for specific statements, compared to other students which had 69% of correct answers for general and 53% for specific statements. In both cases, students of FPB showed

### Table 1. Survey participants and response rate.

<table>
<thead>
<tr>
<th>Participating students</th>
<th>FPB students</th>
<th>SM students</th>
<th>FVM students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penultimate year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(survey participants / total number of students)</td>
<td>12 / 14</td>
<td>18 / 255</td>
<td>52 / 58</td>
</tr>
<tr>
<td>Final year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(survey participants / total number of students)</td>
<td>16 / 26</td>
<td>36 / 292</td>
<td>1 / 66</td>
</tr>
</tbody>
</table>

FPB – Faculty of Pharmacy and Biochemistry (graduate programme Master of Medical biochemistry), SM – School of Medicine, FVM – Faculty of Veterinary Medicine.

### Table 2. Correct answers in the population studied.

<table>
<thead>
<tr>
<th></th>
<th>FPB (%)</th>
<th>SM &amp; FVM (%)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All statements</td>
<td>337 / 392 (86)</td>
<td>927 / 1495 (62)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>General statements*</td>
<td>204 / 224 (91)</td>
<td>590 / 855 (69)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Specific statements†</td>
<td>133 / 168 (79)</td>
<td>341 / 640 (53)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

FPB – Faculty of Pharmacy and Biochemistry (graduate programme Master of Medical biochemistry), SM – School of Medicine, FVM – Faculty of Veterinary Medicine.

Results are presented as the ratio of correct to total answers. *Comprising statements 1, 2, 3, 4, 9, 11, 12 and 13. †Comprising statements 5, 6, 7, 8, 10 and 14. \( P<0.05 \) was considered statistically significant.
more knowledge than SM and FVM students (P < 0.001).

Statements used in the questionnaire and comparison of differences in frequencies of correct answer between FPB group and combined SM and FVM group for each statement is shown in Table 3.

Survey results show, that students from Faculty of Pharmacy and Biochemistry are more conscious about importance of specimen mixing (P = 0.027), prevalence of preanalytical errors (P = 0.001), impact of hemolysis (P = 0.032) and lipemia interferences (P = 0.010), proper choice of anticoagulants

Table 3. Frequency of correct answers according to students’ populations.

<table>
<thead>
<tr>
<th>Survey statement (expected response)</th>
<th>FPB – answers *</th>
<th>SM and FVM – answers *</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sample quality is not dependent on the quality of the sample collection devices. (wrong)</td>
<td>1 / 28 (0.04) 27 / 28 (0.96) 0 / 28 (0.00)</td>
<td>12 / 107 (0.11) 94 / 107 (0.88)</td>
<td>1 / 107 (0.01)</td>
</tr>
<tr>
<td>2. Sample quality is dependent on correct specimen collection technique. (right)</td>
<td>27 / 28 (0.96) 1 / 28 (0.04) 0 / 28 (0.00)</td>
<td>106 / 107 (0.99) 1 / 107 (0.01)</td>
<td>0 / 107 (0.00)</td>
</tr>
<tr>
<td>3. After collection, the sample has to be mixed properly.‡ (right)</td>
<td>23 / 28 (0.82) 5 / 28 (0.18) 0 / 28 (0.00)</td>
<td>63 / 107 (0.59) 39 / 107 (0.36)</td>
<td>5 / 107 (0.05)</td>
</tr>
<tr>
<td>4. In the preanalytical phase of the total testing process occurs prevalent number of errors. (right)</td>
<td>27 / 28 (0.96) 0 / 28 (0.00) 1 / 28 (0.04)</td>
<td>71 / 107 (0.66) 7 / 107 (0.07)</td>
<td>29 / 107 (0.27)</td>
</tr>
<tr>
<td>5. Potassium results from hemolytic samples are reliable. (wrong)</td>
<td>1 / 28 (0.04) 26 / 28 (0.93) 1 / 28 (0.04)</td>
<td>12 / 106 (0.11) 70 / 106 (0.66)</td>
<td>24 / 106 (0.23)</td>
</tr>
<tr>
<td>6. It is possible to remove interference of lipemia before processing the sample on analysers for most of the routine biochemistry analytes. (right)</td>
<td>20 / 28 (0.71) 3 / 28 (0.11) 5 / 28 (0.18)</td>
<td>46 / 107 (0.43) 11 / 107 (0.10)</td>
<td>50 / 107 (0.47)</td>
</tr>
<tr>
<td>7. Coagulation testing is performed in EDTA plasma sample (test tube with the lavender top). (wrong)</td>
<td>4 / 28 (0.14) 23 / 28 (0.82) 1 / 28 (0.04)</td>
<td>42 / 107 (0.39) 50 / 107 (0.47)</td>
<td>15 / 107 (0.14)</td>
</tr>
<tr>
<td>8. Common anticoagulant used for blood gas testing is heparin. (right)</td>
<td>15 / 28 (0.54) 9 / 28 (0.32) 4 / 28 (0.14)</td>
<td>46 / 107 (0.43) 27 / 107 (0.25)</td>
<td>34 / 107 (0.32)</td>
</tr>
<tr>
<td>9. Hemolysis is the most frequent preanalytical interference. (right)</td>
<td>25 / 28 (0.89) 1 / 28 (0.04) 2 / 28 (0.07)</td>
<td>72 / 107 (0.67) 10 / 107 (0.09)</td>
<td>25 / 107 (0.23)</td>
</tr>
<tr>
<td>10. Samples for ammonia analysis are transported at room temperature. (wrong)</td>
<td>0 / 28 (0.00) 24 / 28 (0.86) 4 / 28 (0.14)</td>
<td>23 / 106 (0.22) 34 / 106 (0.32)</td>
<td>49 / 106 (0.46)</td>
</tr>
<tr>
<td>11. Ratio of anticoagulant and blood is not relevant, when blood sampling with anticoagulant is performed. (wrong)</td>
<td>2 / 28 (0.07) 26 / 28 (0.93) 0 / 28 (0.00)</td>
<td>8 / 106 (0.08) 91 / 106 (0.86)</td>
<td>7 / 106 (0.07)</td>
</tr>
<tr>
<td>12. Test results are not dependent on sampling time. (wrong)</td>
<td>0 / 28 (0.00) 28 / 28 (1.00) 0 / 28 (0.00)</td>
<td>43 / 107 (0.40) 62 / 107 (0.58)</td>
<td>2 / 107 (0.02)</td>
</tr>
<tr>
<td>13. Order of draw is not relevant when sample is collected in few test tubes. (wrong)</td>
<td>7 / 28 (0.25) 21 / 28 (0.75) 0 / 28 (0.00)</td>
<td>66 / 107 (0.62) 31 / 107 (0.29)</td>
<td>10 / 107 (0.09)</td>
</tr>
<tr>
<td>14. Complete urine test results are dependent on storage temperature of the sample. (right)</td>
<td>25 / 28 (0.89) 2 / 28 (0.07) 1 / 28 (0.04)</td>
<td>95 / 107 (0.89) 5 / 107 (0.05)</td>
<td>7 / 107 (0.06)</td>
</tr>
</tbody>
</table>

FPB - Faculty of Pharmacy and Biochemistry. SM and FVM - School of Medicine and Faculty of Veterinary Medicine. *Answers offered to survey participants for each statement were “correct”, “incorrect” and “I don’t know”. Data are presented as number of answers/number of total answers (ratio). †Wrong answers and answers “I do not know” were grouped in one category and compared to right answers to the offered statements. Fisher exact test was used for comparison of these two categories. Level of significance was set to 0.05. ‡Statement is not valid for glass BD (Becton, Dickinson) tubes.
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(P = 0.001), transport conditions for ammonia sample (P < 0.001) and order of draw during blood specimen collection (P < 0.001), in comparison with their colleagues from Faculty of Veterinary Medicine and School of Medicine.

Fisher’s exact test for the comparison of frequencies of correct answers between penultimate and final year for School of Medicine showed that there was no difference between these two categories of students. Only one participant from Faculty of Veterinary Medicine was from senior year, so the statistical analysis was not carried out. Significant difference for final and penultimate year students of Faculty of Pharmacy and Biochemistry was found for statement 6 (“It is possible to remove interference of lipemia before processing on analyzer for the most of the routine biochemistry analytes”, P = 0.044), where final year students had higher frequency of correct answers.

Discussion

Results of our survey showed that students from Faculty of Pharmacy and Biochemistry (graduate programme Master of Medical biochemistry) have more knowledge in comparison to their colleagues from School of Medicine and Faculty of Veterinary Medicine. Statements on some specific subjects in preanalytical phase (e.g. ammonia, blood gas testing) showed that all groups of students do not have satisfying level of knowledge.

In order to harmonize and define standards in training programs for clinical chemists across the Europe, Federation of European Societies of Clinical Chemistry (FESCC) carried out the survey on clinical chemistry curriculum in European countries (7). Great heterogeneity in training programs between European countries was found. Framework for training programs in the field of clinical chemistry was defined by the EC4 (European register for specialists in clinical chemistry and laboratory medicine) for underwriting high level of competence of laboratory professionals (8).

Graduate study curriculums of biomedicine in Croatia have different courses regarding laboratory diagnostics. Faculty of Pharmacy and Biochemistry (University of Zagreb) renewed Medical Biochemistry curriculum in accordance with new trends in educational policies (9). On the contrary, Faculty of Veterinary Medicine (University of Zagreb) covers topics about preanalytical phase testing only in one elective course. Medical student education is also being reviewed at many levels, especially considering training in the field of laboratory medicine.

The Academy of Clinical Laboratory Physicians and Scientists (USA) proposed a new curriculum as they revealed that required laboratory medicine courses were conducted in only 57% of United States medical schools (10). In a British survey, 18% to 20% of medical graduates described themselves as “less than competent” in using laboratory testing and more than 20% thought they were less than competent in all diagnostic approaches (11). One survey in veterinary practice found that the majority of respondents were not in compliance with American Society for Veterinary Clinical Pathology guidelines, illustrating the need for improved education of technical staff, veterinary students, and veterinarians (12).

Preanalytical phase of laboratory testing emerged as the most important part of the laboratory practice. Results from the international survey on extra-analytical phase of laboratory testing showed that knowledge and skills of laboratory specialists should be developed in the field of preanalytical laboratory testing (13). The survey of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working group for preanalytical phase on phlebotomy in 28 European countries highlights responsibility and competence of the laboratory specialist in implementation of the relevant guidelines and education of the personnel (14).

Our survey study was carried out in student population of penultimate and final year from Faculty of Pharmacy and Biochemistry, University of Zagreb, as well as from the other biomedical faculties. The aforementioned surveys were performed in a population of professionals directly or indirectly involved in laboratory practice. Survey results point to the need for improvement of education pro-
gram in the field of laboratory medicine. To the best of our knowledge, there are no similar survey studies on graduate biomedicine student population. Recently, one survey study on general skills and competencies in the field of biochemistry was carried out in population of postgraduate students, educators and industry (15). In contrast to our survey, it was not carried on student population exclusively and did not investigate level of knowledge in specific field of biomedical science.

Most consistent results were found in population of Faculty of Pharmacy and Biochemistry students. Interestingly, there were no differences in frequency of correct answers when penultimate and final year students of Faculty of Pharmacy and Biochemistry were compared, except one statement related to lipemia interference. This difference could be due to topics covered by the program/curriculum for the final year and the program of training for students in hospital laboratory.

Heterogeneity of the survey results for students from School of Medicine and Faculty of Veterinary Medicine indicates the lack in the respective curriculum for laboratory diagnostics. It is extremely important that all biomedical professionals adopt skills which will guarantee high specimen quality and consequently, most reliable analysis result. Organization of laboratory service involves specimen collection, storage and transport to core laboratory from wards, physician’s practices, infirmaries, etc. For that reason education of all biomedical professionals in the field of preanalytics should be stressed.

Since the statements of the questionnaire reflect our personal impressions about various most problematic preanalytical issues, some uncertainties could have been misleading to survey participants. Statement 7 implies that lavender top stands for EDTA tube, which is indeed true only for BD (Becton, Dickinson and Company) and Greiner tubes, but not for Sarstedt EDTA tubes (Sarstedt AG & Co, Nümbrecht, Germany) which have green tube closures. We recognize that this question might have been interpreted differently in some other countries, but in Croatian laboratories, BD and Greiner are the most prevalent tube distributors and this is why we presumed that majority (if not all) participants would have a correct understanding of the statement. Moreover, the color of the tube was added only in parenthesis and the additive was clearly stated as EDTA minimizing the potential for misunderstanding.

In the 11th statement on importance of correct ratio between anticoagulant and blood, we wanted to investigate the awareness of students on that topic. The selection of appropriate anticoagulant, its form (liquid, lyophilized) and final concentration in blood sample have direct impact on analyte concentration (16). More precisely, we wanted to investigate the participants’ awareness about the importance of drawing blood in tube up to the mark for all tests performed in whole blood and plasma (EDTA, oxalate, heparin samples). In that way ratio between blood and anticoagulant is optimal which leads to correct and reliable results. Maybe our intention was not clear and understandable from the way we formulated statement 11 and that might have effect on how students answered this question. This could be a possible limitation of our study.

Likewise, our statement number 12, about sampling time pointed at the extensive number of analytes for which is the recommended sampling time in the morning. According to the Working Group on Preanalytical Phase (WG-PA) of EFLM, blood for all tests should be drawn from the 07.00 to 09.00 a.m. (17). Possible limitation can be that we have not mentioned precise time of blood drawing which might have been misleading to the participants, therefore their answers might have been different.

Statement 13 was related to the order of draw during the sampling procedure. There are many recent publications opposing or supporting current guidelines from Clinical and Laboratory Standards Institute (CLSI), rendering this part of the venous blood sampling procedure controversial (18,19). Still, recommendations of the Working group for preanalytical phase of the Croatian Society of Medical Biochemistry and Laboratory Medicine point out that compliance to the order of draw is an important step of the sampling procedure (20).
As our questionnaire was adapted to the current recommendations in Croatian laboratory practice, we presumed that participants would have a correct understanding of the statement.

With statement 14 we wanted to find out the level of awareness on urine sample stability and impact of storage temperature on routine urine analysis (21). The expression “complete urine test results” was used for urine dipstick and microscopic sediment analysis. It is a Croatian term widely used among health care workers in clinical practice in Croatia. Even though it might seem unclear to non-Croatian participants, our survey was designed for future health care workers who were familiar with this expression.

When this study was planned and prepared guidelines for creating and reporting surveys were available (16-20). Unfortunately, we did not consult any of these guidelines and that might have affected the quality of our data and our results. Furthermore, population of Faculty of Pharmacy and Biochemistry students was rather small in relation to the other two tested groups, so the comparison was disproportional. This disproportion arises from the limited number of students admitted to FPB study program of medical biochemistry (only 20 yearly). Additionally, survey was conducted in period from June to September of 2014 and covered students of penultimate and final year at that time frame, limiting the possibility to raise the response rate. Also, the response rate of the students from the School of Medicine was low. As only one student from senior year from Faculty of Veterinary Medicine participated in survey, the statistical analysis for comparison of frequencies of correct answers between penultimate and final year students was not carried out. These are the possible limitations of our study.

In conclusion, survey results showed that students from Faculty of Pharmacy and Biochemistry are more conscious on the importance of preanalytical phase of testing in comparison with their colleagues from other biomedicine faculties and no difference in knowledge between penultimate and final year of the same faculty was found. Implementation of education programs concerning preanalytical phase of laboratory testing is needed in order to improve patient care.

Acknowledgment

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Potential conflict of interest

None declared.

References

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