A Web-based Laboratory Subsystem for Serum Antibody Specificity Analysis in a Laboratory Information System

Vanco Cabukovski
Faculty of Natural Sciences and Mathematics
Ss. Cyril and Methodius University
1000 Skopje, Republic of Macedonia

Roman Golubovski
Faculty of Natural Sciences and Mathematics
Ss. Cyril and Methodius University
1000 Skopje, Republic of Macedonia

ABSTRACT
In this paper a web-based laboratory subsystem for serum antibody specificity analysis is presented. This subsystem is an efficient laboratory tool for detection and analysis of serum antibody specificity. It is shown that the subsystem can provide satisfactory way for the serum analysis.

General Terms
HLA antigens, Antibody specificity, Serum quality, Web-based computer program, Detection, Terasaki's microplate.

Keywords
Web-based laboratory system; serum antibody specificity analysis.

1. INTRODUCTION
Alloimunisation is a consequence of pregnancy, blood transfusion or transplantation of tissues and organs. The detection of antibodies and their specificity is important, especially for patients who receive blood transfusion frequently and for patients with end stage renal diseases (ESRD) waiting for transplantation [1, 2, 3].

Determination of antibody specificity enables avoidance of risk of transfusions and incompatible transplantation [4, 5, 6]. The polymorphism of Major Histocompatibility Complex (MHC) e.g. HLA antigens and cross-reactions among them makes this analysis difficult [1]. So, laboratory computerizations described in [2, 3, 7, 8, 11] simplifies this analysis. The computerized laboratory systems give fast, precise, complete and objective results.

This paper proposes a web-based laboratory subsystem for serum antibody specificity analysis developed in Java with MySQL embedded engine.

2. THE FUNCTIONALITY OF THE SUBSYSTEM
A web-based laboratory subsystem for serum antibody specific analysis is based on a computer program for detection of antibody specificity in serum analysis (serum quality) developed in Java with MySQL embedded engine.

The developed web-based laboratory subsystem consists of four main functional parts covering the following laboratory activities:

- Battery description;

- Recording the serum reactions with a panel;

- Calculation of the serum quantity and quality;

- Reporting the serum results.

All these activities are enabled through a specially designed GUI offering an easy user manipulation. The UI is user friendly oriented so, that the data manipulation like insertion, editing, deletion, etc. are make in a similar way as manually work.

Each of these functional parts is described below.

2.1 Battery Description
One battery contains the arrangement of 30 serums taken from patients for the serum antibody analysis. Each serum is presented by a serum code which contains a letter and three or four digit integer numbers (like H-745, H-746, I-967, B-2119, etc.). The letter used is internal code for the patient's status or patient's disease and the number is an internal description of the serum.

For each serum two vertical neighboring cells of the microplate are being dedicated. So, 60 cells of the microplate are covered by 30 different serums taken from 30 different patients.

Each battery is named with four digit number enabling the full battery access. In Figure 1 is given a battery description screen consisting of battery number and the date of battery description. On the right side of the screen is given a graphical grid representing graphically the microplate for lymphocytotoxicity test e.g. Terasaki's microplate [10,12].
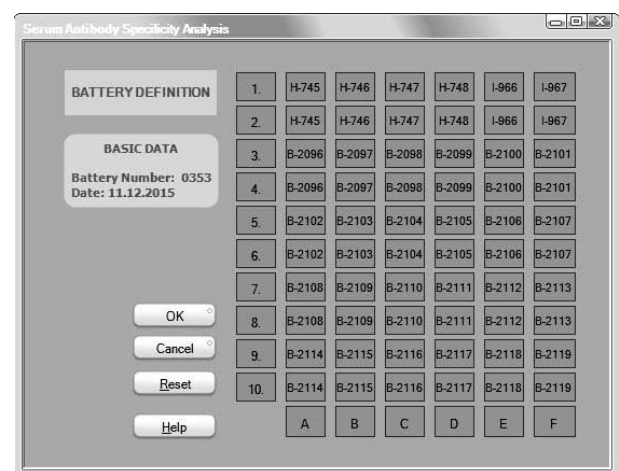

Figure 1. Battery definition screen

2.2 Recording the Serum Reactions with a Panel
The serum's cytotoxicity is tested with special analysis of all their reactions with the lymphocytes from more than 40 HLA defined persons. All these persons for a specific battery of serums define a set of different panels. In a subsystem

developed all reactions with the set of panels, for each serum from a battery are recorded. In Figure 2 the screen for recording of the serum reactions with a panel is shown.
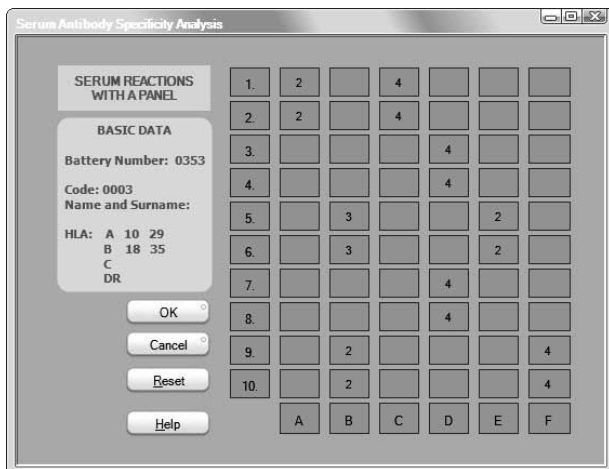

Figure 2. Serum reactions with a panel screen

From the set of panels for each person the following basic data are necessary: the battery number, code, name and surname and the determined HLA – A, B, C and DR antigens. In the right side of the screen a graphic representation of the serum reactions with a panel, for any person, is shown in a same order as in Terasaki's microplate from the lymphocytotoxicity test (Figure 2).

2.3 The Serum Quantity and Quality Calculations

The serum quantity and quality are evaluated through a mathematical calculations consisting of the following data: number of antigens occurred in a set of panels for a battery (NAg), the total number of reactions for each serum in a battery (Nrs) and the number of reactions for a serum-antigen combination (Nrsa).

The serum quantity is evaluated by the formula: $Sq=Nrs/N$, where N is a total number of panels tested with a battery.

The serum quality is evaluated by a standard Chi-square test for 2x2 contingency tables using the formulas presented below for calculation of each cell's frequency:

Nrsa	Nrs – Nrsa
NAg-Nrsa	N-(NAg+Nrs)+Nrsa

In this way the quantity of serum reactivity with the set of panels and the antibody specificity are determined by the subsystem. Results are presented in a list as shown in Figure 3.

Two different calculation models for serum quantity and quality evaluation are incorporated in a subsystem. The first model observes each antigen alone and the second model observes complex antigens (public and splits). The user of the subsystem can always select one of these two models for serum quantity and quality calculation.
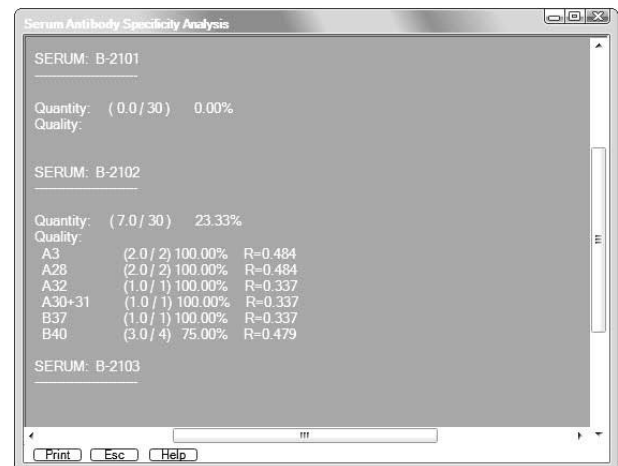

Figure 3. The quantity of serum reactivity and the antibody specificity screen

2.4 Reporting the Serum Results

The results from serum analysis are available for each serum (patient). Two types of result reports concerning each of the calculation models are available by the subsystem. The report is consisting of antigen specificity (quantity and quality) for a selected serum, calculated by the first or second calculation model applied. The report can be printed out in an appropriate form as a final result for a patient as it is shown in Figure 4.

3. SOME LIMITATIONS OF THE SUBSYSTEM

The so-called PRA (Panel-Reactive Antibody) represents a semiquantitative estimate of the degree of HLA sensitization. It is calculated as the percentage of an HLA panel that reacts with a serum. Patients with >80% PRA are considered highly sensitized and for them it is difficult to find crossmatch-negative donors [12]. The analysis of serum reactivity patterns with HLA phenotyped panels in cell-based and solid-phase assays is primarily done with 2×2 table statistical methods such as chi square to identify antigens and epitopes with significant correlations. This method is of limited value for >80% PRA serums.

4. FUTURE DEVELOPMENT OF THE SUBSYSTEM

One of the possible future additions to this software is hardware automatization based on on-line computer connection with a microscope. In [9] and [13] a new flow cytometry method for cross-match evaluation has been proposed. This method allows a better detection of weak positive reactions than the light microscopy. The program will be upgraded with this method. For this purpose a new functional part of the program should be developed which will provide automatic recognition of the positive reactions.

The other future development of the program is a selection of acceptable and unacceptable donor mismatches in kidney transplantation for patients with high rate of alloimmunisation and on-line connection with TRANSPRIORI subsystem for selection of candidates for cadaveric renal transplantation [4].
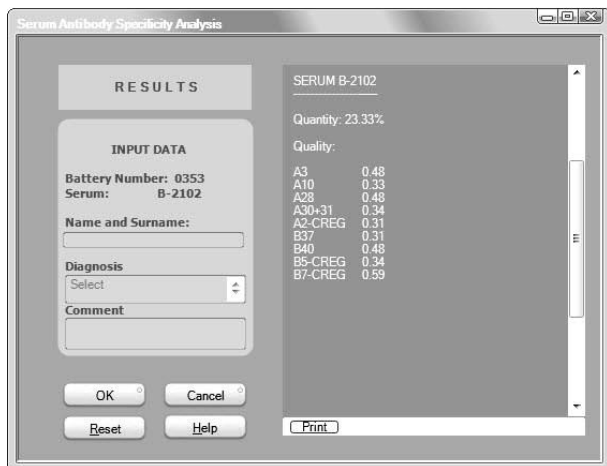
Figure 4. Serum analysis patient's results screen

5. CONCLUSION

In the paper a web-based laboratory subsystem for serum antibody specificity analysis is presented. It is shown that the subsystem can provide an efficient way for this type of laboratory analysis. The program user interface is user-friendly oriented and data operation and manipulation are simplified in comparison with the manual laboratory work. We are continuing our research in direction of full automatization of this subsystem.

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