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Automation of laboratory tests of blood donors in the field of blood-borne infectious agents in the context of own experience of Regional Center for Blood Donation and Blood Treatment in Kalisz

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Summary

The paper discusses the importance of automation of respective stages of laboratory testing for blood-borne infectious agents at the Regional Blood Transfusion Center. Based on our own experience, the gradual development of the laboratory was described, the solutions used were presented and their advantages were pointed out. The aim of the study was to show that technological progress and the associated automation are essential elements in ensuring reliable research results which translates into strengthening the safety of blood and blood components.

Keywords: automation, blood donation, laboratory test reliability, blood-borne infectious agents

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Introduction

The Laboratory of Blood-Borne Infectious Agents at the Regional Blood Transfusion Center (RCKiK) in Kalisz performs serological and molecular marker donor screening for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and anti-Tryponema pallidum (T. pallidum) and therefore contributes to strengthening the safety of blood and blood components. Accordingly, the tests and equipment used must meet strict requirements [1–3].

Technological advancement, development of newer solutions, and the special emphasis on test quality render the process of automation of laboratory workflow indispensable. Automation allows standardizing some procedures and effectively taking advantage of the opportunities offered by current technological achievements [4].

A laboratory process that allows obtaining reliable test results is divided into 3 stages: pre-analytical, analytical and postanalytical. Each stage is susceptible to errors that may adversely affect the final result. With this in mind, it is important to implement measures to minimize the risk of errors. It has been proven that the majority of issues are associated with human error, therefore it is important to ensure that the available technology supports and improves routine laboratory performance [5].

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Pre-analytical phase — preparation of material for testing

The pre-analytical phase is fundamental for the whole testing process. The main stages are: preparation of samples (centrifugation included), identification of the tested sample, appropriate qualitative and quantitative evaluation of the test material, and supply of the proper material for testing [4, 6].

Initially, all test tubes were centrifuged manually. Following centrifugation, the employee preparing the material had to assess whether the material was suitable for testing, decapping the test tubes and place them in appropriate racks. A considerable amount of time and attention had to be devoted to this stage because the number of tubes was quite significant (3 different types from each donor) and the number of places in the centrifuges was limited (initially 60 places for molecular biology research, 64 places for serology). Moreover, it was important to maintain correct centrifugation parameters, as there was a possibility of their modification.

In order to standardize the procedure and to shorten the time for preparation of the testing material the decision was made to lease a cobas® p512 preanalytical system, which was over time expanded to include a cobas® p471 centrifuge module. Simultaneous centrifugation of different types of samples ensures uniform, arbitrarily imposed centrifugation parameters. The centrifuge and sorter are connected to each other. After the tubes are properly centrifuged, they are automatically transported to the sorter, which recognizes the samples being tested. It makes a qualitative (for the presence of lipemia, hemolysis, and icterus) and quantitative (the volume of test material) evaluation and decaps those intended for current testing, and places them in appropriately programmed racks. Specimens that do not meet requirements are set aside for the user to decide whether the material is suitable for testing.

Moreover, within the device, images of each sample are stored, captured by a high-resolution camera, along with information regarding the path of individual samples on the device. In addition, the cobas® p512 identifies samples that are flagged incorrectly to make sure that tests are made on correct material. A preprogrammed algorithm, which includes the tube's catalogue and flag number, allows the system to evaluate the match and, if an error is identified, the sample is set aside in a strictly programmed location.

The system is operated dynamically using the laboratory information system (LIS). Based on the received orders, the tubes are either decapped or sorted into designated locations [6]. Thanks to the connection between the cobas® p512 device and the LIS, after scanning the barcode affixed to the tube, information about the time and date of this action (sample reception by the system) is recorded in the LIS. Information about any qualitative or quantitative errors can also be transmitted to the LIS after proper configuration.

The development of appropriate sorter modes, depending on the laboratory requirements, significantly facilitates work flow and allows customizing the system. The configurations may be adjusted to the changes in the laboratory's mode of operation at any time.

In our laboratory, the cobas® p512 preanalytical system with a cobas® p471 centrifuge is connected by CCM lines to the analyzers which perform serological tests, namely two cobas® 8000 e602 and a cobas® 6800 analyzer which performs molecular tests. This allows transporting appropriate samples directly to the decks of the respective units. With this option, it is also possible to fully automate molecular testing for single donations. The samples are then decapped in the sorter, transported by CCM lines (cobas® connection modules) and collected on cobas® 6800 analyzer, which automatically starts the process after reaching the maximum number of samples for a given run (91 samples) or after 2 hours from loading the first sample onto the platform. The next run starts approximately 1.5 hours later and the user's role is merely to replenish consumables.

Thus, the pre-analytical phase seamlessly merges into the analytical phase. The benefit of this solution lies in the standardization of this testing stage. All samples entering the pre-analytical system are treated in the same specific predefined way.

It is the user who defines which samples are to be rejected as non-compliant. At the same time, the final decision on questionable samples rests with the person performing the tests, which gives flexibility in decision-making. By reducing the time required for this stage, more attention may be focused on the analytical phase itself — preparing the analyzers for testing, analyzing the results of quality control or, if necessary, analyzing results that deviate from accepted standards. More attention may be focused on documentation.

Sample identification by the sorter also eliminates human error associated with incorrect barcode labeling of the test material.

An automatic centrifugation program ensures that all tubes that come on board are centrifuged at the right time at the correct centrifugation parameters.

Analytical phase — performance of tests

Once the test material has been properly prepared the next stage in the entire research process is conducting the appropriate tests.

Molecular biology — HIV RNA, HBV DNA and HCV RNA testing

Successive generations of analyzers performing molecular biology tests, up to the cobas® 6800, have allowed for shorter testing times, increased efficiency, reduced staff contact with potentially infectious biological material, and increased availability of materials for testing. Previously, it was only serum, but now there is also the possibility of conducting tests from plasma. It has also become possible to establish a single laboratory for molecular as well as serological tests. This was so far impossible due to the higher risk of contamination and the requirement of following a strictly defined organization of work which included a separate area for the molecular biology laboratory. Moreover, the laboratory space was divided into three areas: for preparing the testing material, for isolating the testing material and for direct amplification and detection of genetic material. Movement between the three areas required change of protective clothing.

As concerns testing donor molecular markers in plasma pools, it is also important that the pooling equipment (in tour cobas® p680) and the equipment which performs the testing (cobas® 6800) are communicated with each other, and the role of the staff is limited to loading the tubes, reagents and consumables onto the appropriate analyzer decks [7, 8].

Another important aspect is that the quality controls are added only on board of the cobas® 6800 where the tests are performed which minimizes contamination of samples, the pooling equipment and the laboratory area with HIV, HBV, HCV genetic material. Moreover, the used cassettes with controls are not discarded by the analyzer into solid waste containers, but are disposed of by the user which contributes to protecting the laboratory from contamination with genetic material.

The entire process — from creating the test pool to obtaining results — is monitored, and any errors that may occur are appropriately reported. In the event that repeat assays are necessary, after loading the samples onto the cobas® p 680 pooling device, the samples in which repeat assays need to be performed are automatically selected [8].

Archiving of tested material

Another issue is the necessity of creating an archive for each tested sample [1, 9]. In our laboratory, archive plates are formed at the pool-preparation stage by the cobas® p680 analyzer. The software informs on which plate and in which position the archive sample was placed, as well as on the volume of archived material (correctly, the volume should be ≥ 1 ml) [1]. When the sample requires retesting, it can easily be a found and identified [1, 8].

Assays

The multiplex cobas® MPX assay used for routine molecular testing has also been adapted to current knowledge related to virus infection variability, in particular HIV, so that two regions of HIV RNA genetic material are now tested as safeguard against the so-called escape mutants [3]. Moreover, multiplex test has significantly higher sensitivity to HIV and HBV than the tests used so far. For the currently used cobas® MPX test, the analytical sensitivity [95% level of detection (LOD, limit of detection)] according to the manufacturer's leaflet is, respectively, for HIV-1 RNA — 25.7 IU/mL, for HCV RNA — 7.0 IU/ml, and for HBV DNA — 1.4 IU/mL, while for the previously used multiplex test Cobas® Taqscreen MPX version 2.0. these values are: for HIV-1 RNA — 50.3 IU/mL, for HCV RNA — 6.8 IU/mL, and for HBV DNA — 2.3 IU/mL [10–12]. Higher sensitivity of HBV DNA detection is particularly important for blood donation as it reduces the risk of transfusion transmitted infections [13].

Methods

From a diagnostic point of view, it is also important that the currently used real-time polymerase chain reaction (RT-PCR) has many safeguards against incorrect results. Strictly defined rules for the validity of quality control results, internal control of tests, and enzymatic protection against nonspecific reaction products significantly contribute to increasing the safety of the tests used [12].

The proper labeling, starting from the isolation of genetic material through its amplification

and detection, occurs in a single device, ensuring that each of the individual stages is guaranteed the appropriate conditions, and the analyzer's software oversees each subsequent step until the result is obtained.

Serological screening — HBsAg, anti-HCV, anti-HIV1+2, anti-T. pallidum

Serological marker screening has also evolved over the years: from manual (microscopic examination for anti-T. pallidum antibodies) through semi-automated units to fully automatic analyzers — the cobas® 8000 e602.

Modern instruments provide objective, reproducible reading and interpretation of test results, eliminate the risk of confusing test samples during preparation of test material and, most importantly, are equipped with an internal quality control system. In the event that the results of the company's controls fall outside the defined range of standard deviations, the reagent is blocked, the result is labeled accordingly [14, 15]. Additionally, in the event of a change in the batch of reagent used, calibration is necessary, which serves as an additional safeguard against using invalidated reagents [15]. Above cutoff value results that have to be repeated are also clearly flagged, as is any information about problems with pipetting samples/reagents.

The cobas® 8000 e602 makes it possible, from the data manager (the software that manages the output generated by the analyzer related to quality control tests and donor results), to analyze quality control Levey-Jennings charts and thus control whether the method meets the assumptions made by the analyzer and to react when certain rules are violated. Quality control results are subject to two Westgard rules. The first rule 1 × 2SD [a single assay exceeds a level of 2 standard deviations (SD, *standard deviation*)] is a warning rule, indicating that an accidental error has occurred. If this rule is violated again, it is necessary to implement appropriate measures to clarify the cause of the situation. The second rule 1 × 3SD (a single determination exceeding the level of 3 standard deviations), if violated, results in the blocking of the test being used until correct determination results (falling within the range of mean ± 2 SD) are obtained and manually confirmed for accuracy from the *Data Manager* level.

Applied tests

The process of performing serological tests using the apparatus has also significantly shortened over the years. Currently, the longest determination is the HIV test and takes 28 minutes, which, compared to the previous analyzer, where the longest test took 58 minutes, has significantly reduced the waiting time for a set of donor serological results. Another added value is that the fourth-generation Elecsys® HIV Combi PT test detects the p24 antigen in addition to antibodies, which appears much earlier, a few days after infection. This contributes to the shortening of the serological window period [3, 16].

All of the above facilities related to the technological advancement of the analyzers, which are located in the authors' laboratory, significantly reduce testing time, allow tracking the test samples and facilitate control over the reliability of the results [1–3].

Postanalytical phase — analysis of obtained results

After a complete set of serological tests and nucleic acid testing (NAT, nucleic acid testing) is performed, the results with a status of "non-reactive" for serological marker assays and "not detected" for molecular marker assays are automatically sent to the laboratory information system (LIS), where they are validated by the laboratory diagnostician for release of blood and blood components for clinical use and fractionation [1]. In the event that repeat determinations are required (a reactive result is obtained in serological tests), all subsequent tests are transferred to the LIS and, depending on the constellation of results obtained, and the laboratory diagnostician determines the final test result. In the case of molecular biology tests, the reactive pool is withheld for clarification, and once the results are obtained for the individual donations included in the pool, they are forwarded to the LIS.

Information about the date, time of the test, the person performing the test, the apparatus on which the assay was performed, among other things, is also sent to the ICT system. This is the integration of the analytical phase with the post-analytical phase, which is also automated, and through which it is possible to view the donor's complete test results, determine the final result and accept the results obtained.

Supervision of analyzers and laboratory equipment

In order to ensure the proper functioning of all the elements mentioned so far, it is necessary

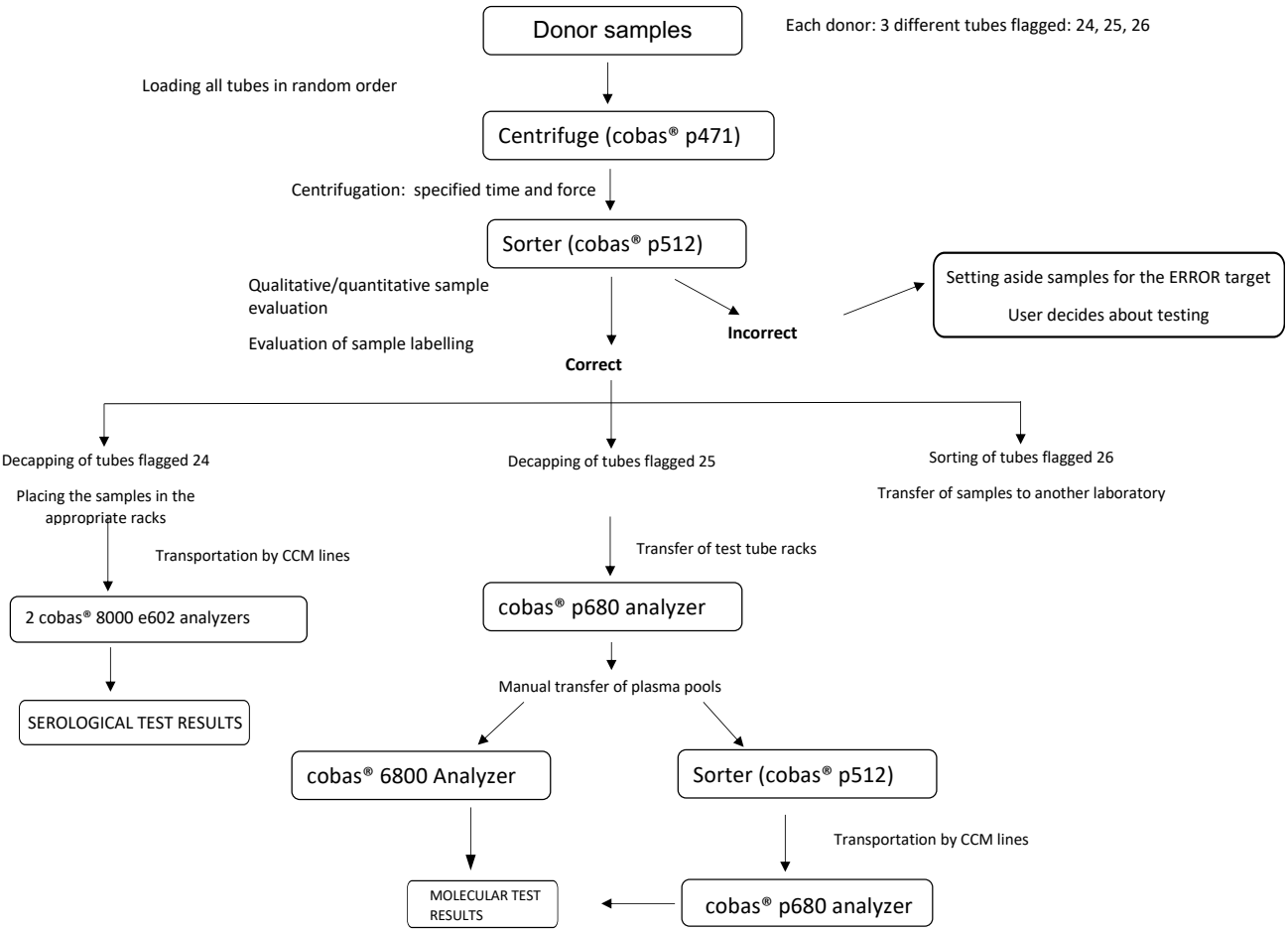

Figure 1. The work flow for virological testing at the Regional Blood Transfusion Center in Kalisz

to have software that can manage the operation of individual instruments and track the fate of samples coming to the laboratory for testing markers of infection: HBV, HCV, HIV, T. pallidum bacteria in donors in accordance with current regulations [1].

The final step in the automation of the authors' laboratory related to the performance of tests was the implementation of a program that integrates all the instruments in the laboratory — cobas® Infinity central lab. From its interface, it is possible to have oversight of what is going on in the lab: which instruments are running, what are the results of quality control, it is possible to follow the path of individual samples (pre-analytical and analytical phases) and whether a particular sample already has a set of assays, as well as to check whether a particular tube even made it to the assay. It is also possible to mask, meaning to temporarily disable, individual modules from routine operation — such as the cobas® 8000 e 602 analyzers and the cobas® 6800 analyzer — as needed. This might be necessary for maintenance tasks, periodic inspections, repairs, conducting reagent qualifications, or investigating irregularities related to quality control of the tests.

Laboratory supporting systems

Another system supporting the lab's work is navify® Inventory, a virtual warehouse application that manages supplies and inventory levels in the laboratory. It allows automatic generation of orders based on a defined schedule. The generated list can be automatically sent to Roche or the user can accept it on his own, for example, after completing the items at his own discretion. Additionally, at any time, if needed, users can create their own orders for specific items.

The program also keeps an eye on the expiration dates of reagents and the order in which they

Table 1. Elements of quality control at different stages of the testing process

Phase	Elements of quality control	Proceedings
Pre-analytical phase	Qualitative evaluation of test material: lipemia, hemolysis, icterus	Deposit the non-compliant tubes in the designated place
	Quantitative evaluation of test material: sample volume	
	Evaluation of the correctness of barcode labeling on the tube	User decides to perform tests
Analytical phase — serological tests	Performance of daily quality control and analysis of results	Test blocked if values > 3 standard deviations
	Calibration of new reagent batch/series	New batch of reagent blocked until proper calibration
	Reporting any error related to the analyzed sample: • clot detected • incorrectly affixed barcode • damaged barcode • presence of air bubbles	Repeat the test
Analytical phase — molecular tests	Error reporting related to the analyzed sample: clot	Repeat the test
	Evaluation of controls performed in each test run	Incorrect result for the control, cancellation of the test run or cancellation of individual pools
Post-analytical phase	Clear labeling of samples with above cut-off values	Repeat the tests; final decision
Work support system — cobas® infinity central lab	Management of the analyzers	Masking individual analyzers in the analytical phase (incorrect quality control results, need for maintenance, equipment malfunction)
		Insight into current results for individual donations
		Tracking of the individual tubes

are used, and provides the ability to create reports for reporting purposes, for example, of the number of materials accepted/used in a given time interval.

The application also allows you to transfer individual goods between the created warehouses. It is also possible to prepare a list of reagents with a short shelf life, such as 14 days, and distribute them to where they are sure to be used. In addition to this, it is possible to track the status of a placed order, and delivery acceptances are made using handy barcode readers with just a few clicks. It also allows downloading and returning goods to the virtual warehouse.

The entire system facilitates inventory management and efficient use of reagents.

Another functionality launched by Roche is the navify® portal, through which it is possible to make service requests and view the entire repair history of a given analyzer. In addition, thanks to the eLabDoc tab, it is possible to access online the current instructions for use of reagents, controls, calibrators, declarations of conformity and safety data sheets for substances or hazardous mixtures. The "My Orders" tab allows you to follow the path of your order and access related documentation (release documents, invoices).

Conclusions

As shown in Figure 1, the entire automation process in our laboratory met expectations — it allowed to implement modern solutions to raise the level of laboratory performance which — given its specificity — is of utmost importance.

Undeniable benefits of comprehensive automation of individual laboratory work stages include: increased quality control over laboratory operations in accordance with Table 1, shortened waiting time for results, the ability to manage individual system components to streamline operations, and documentation of each stage, from sample reception to testing, through assessment of the quality of the material under examination and performing assays, to issuing test results.

Table 2. Stages of the testing process of 96 donations (266 tubes) at the Regional Blood Transfusion Center in Kalisz

Phase	Components	Time	Performance of the equipment declared by the manufacturer
Pre-analytical phase	Loading test tubes into the centrifuge (cobas® p471)	1 hour	Up to 247 tubes/1 hr*
	Spinning		Up to 247 tubes/1 hr*
	Unloading and sorting of test tubes (cobas® p512)		Up to 1400 tubes/1 hr [18]
Analytical phase: 2 cobas® 8000 e602 analyzers	Scanning the barcode of the first donation	2 hours	340 tests/1 hr 85 donations/1 hr**
	Completion of one batch		
Analytical phase: cobas® p680 analyzer	Preparation of plasma pool and archive:	30 minutes	192 donations/1 hr#
Analytical phase: cobas® 6800 analyzer	Completion of testing of one batch:	2 hours 40 minutes	546 donations/3.5 hrs##
Post-analytical phase	Analysis and authorization of test results:	20 minutes	
Duration of the whole diagnostic process&		4 hours 30 minutes	

Assuming that: *centrifugation lasts 10 min [17]; **4 tests per donor [18]; #determined from own experience; ##testing pools of 6 [19]; &assuming parallel performance of serological and molecular tests

The entire testing process of 96 donations (266 samples) lasts 4.5 hours, according to Table 2.

On the one hand, the example described makes it perfectly clear that automation is a valuable and necessary path of technological development. It enables the use of technological advances and solutions to ensure reliable test results. At the same time, it makes it possible to meet the growing needs for a cost-effective approach to laboratory work. This applies to both possible greater flexibility in hiring personnel (over the past few years, the number of employed staff in the authors' laboratory has decreased) and the efficient use of reagents and consumables.However, on the other hand, it should be emphasized that automation should not exclude human presence in the entire process of test performance. On the contrary, it is meant to support work, make the process as standardized as possible, but leave the final decision on interpretation to appropriately qualified personnel.

The ability to configure individual elements of the entire system according to one's own needs allows these solutions to be adapted to different situations. It is also important that by implementing such systems into routine work, one can observe how everything functions as a whole, make necessary adjustments, and think about what else could be changed to improve the workflow.

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