Automation of Blood Donor Testing for Infectious Diseases

Annette Schuller · Willy Kurt Roth

GFE Blut mbH, Frankfurt/M., Germany

Key Words

Automation · Blood donor screening · Serological testing · NAT

Summary

The early recognised risk of transmitting infectious diseases by blood components and the consequent introduction of mandatory serological testing of blood donations for infectious diseases has pushed the development of various automated serological testing systems. As correct performance of pre- and post-analytical work steps is essential for obtaining valid test results, the need for standardisation of these processes has led to the development of several supporting front-end systems. Since the recent introduction of mandatory NAT testing, first approaches for automation of NAT have already been made by different manufacturers. As the currently available systems do not meet exactly their needs, several German blood donation services are currently developing an own automated NAT testing system. Automated testing systems yield several advantages compared to manual testing methods. Besides the standardisation and complete documentation of processes, automated systems reduce the infection risk for the staff and help to avoid typical human failures. In this review the features of representative automated serological and NAT testing systems are described exemplarily. Furthermore, established automated front-end systems are presented and compared with each other. Perspectives of automation of blood donor testing for infectious diseases like installation of laboratory assembly lines and integration of serological and NAT testing are pointed out.

Schlüsselwörter

Automation · Blutspender-Screening · Infektionsserologie · NAT

Zusammenfassung

Das bereits frühzeitig erkannte Risiko der Infektionsübertragung durch Blutprodukte und die darauffolgende Einführung einer verpflichtenden infektionsserologischen Testung von Blutspenden hat die Entwicklung verschiedener automatisierter serologischer Testsysteme vorangetrieben. Da die korrekte Durchführung prä- und post-analytischer Arbeitsschritte einen essentiellen Faktor für die Erzielung valider Testergebnisse darstellt, hat das Bedürfnis zur Standardisierung dieser Prozesse zur Entwicklung mehrerer unterstützender Front-End-Systeme geführt. Nach der Einführung einer verpflichtenden NAT-Testung wurden von einigen Herstellern bereits erste Entwicklungen hinsichtlich einer Automation der NAT unternommen. Da die gegenwärtig verfügbaren Systeme den Bedürfnissen der deutschen Blutspendedienste nicht vollständig gerecht werden, entwickeln einige Blutspendedienste derzeit ein eigenes automatisiertes NAT-Testsystem. Automatisierte Testsysteme weisen gegenüber manuellen Testmethoden mehrere Vorteile auf. Neben der Standardisierung und der vollständigen Dokumentation aller Prozesse reduziert der Einsatz automatisierter Systeme das Infektionsrisiko für das Personal und dient der Vermeidung typischer menschlicher Fehler. In der vorliegenden Übersicht werden die Charakteristika automatisierter serologischer und NAT-Testsysteme anhand einiger repräsentativer Beispiele beschrieben. Darüber hinaus werden etablierte automatisierte Front-End-Systeme vorgestellt und miteinander verglichen. Perspektiven für die Automation der Testung von Blutspendern auf Infektionskrankheiten wie die Einrichtung von Laborstraßen oder die Verflechtung von serologischer und NAT-Testung werden aufgezeigt.

Introduction

The directives based on the German Transfusion Law, in conjunction with the votes of the Advisory Board on Blood Affairs, request the testing of all blood donations for anti-HIV-1/-2, anti-HCV, HBsAg, anti-HBc, syphilis (TPHA, TPPA or EIA), HCV-NAT and HIV-1-NAT. In addition, HBV-NAT is voluntarily performed by many blood donation services. For the transfusion of immunocompromised patients, testing of blood components for anti-CMV is also strongly recommended besides the fact that general leucodepletion has been introduced recently. In the run-up to allogeneic peripheral blood stem cell collection, the donor is tested additionally for anti-CMV and anti-HBs and often voluntarily for anti-HTLV-I/-II, anti-HAV IgG and IgM as well as for various other infection parameters. Plasma fractionators generally demand testing of plasma pools for HAV and parvovirus B19.

Automation in serological and NAT testing of blood donors for infectious diseases yields several advantages compared to manual testing methods. With the application of an automated system the infection risk for the staff decreases while at the same time human failures like mixing up of samples, pipetting errors or impreciseness, carry-over, etc. can no longer occur. All processes, including sample sorting, decapping, pooling and/or aliquoting, the performance of assays, the archiving of samples, the interpretation of test results, and the complete documentation can be fully controlled within an automated system. With regard to the accreditation of laboratories, the wholly controllable and transparent workflow provided by automated systems is a major benefit. Moreover, automation helps to straighten the routine workflow, to standardise the testing processes, to ease the workload of the staff, and to optimise the relation of costs and performance.

For the automated serological testing of blood donors for infectious diseases there are already a variety of different systems available. With regard to NAT testing some promising approaches are currently being finalised. Besides the actual testing systems various auxiliary and supporting systems, e.g. for decapping of tubes, aliquoting of samples, sample distribution, sample archiving and recapping of tubes, have been developed.

The automated serological testing systems can be classified into two major types: 'closed' systems which can only process special tests directly designed by the manufacturer and 'open' systems which can process various tests independent of the manufacturer. Furthermore, the capability of the system to process all mandatory assays, the availability of state-of-the-art tests for the respective automated system and all facultative assays performed by the user are essential factors which have to be taken into consideration when evaluating automated testing systems. Another important aspect is the flexibility concerning sample tube types, i.e. the flexibility of the test system to process sample tubes from different manufacturers and/or different sample tube sizes. The sample throughput of

the system compared to the sample numbers which have to be processed in a given time also has to be considered. Factors which improve the user convenience, like centrifugable sample racks or compatibility of sample racks with supporting systems, computerised support of quality control and system maintenance, capability of bi-directional data exchange with the laboratory software, and extent of electronic documentation have to be taken into account.

In the following, different automated systems for the serological testing of blood donors for infectious diseases will be presented with special regard to the above mentioned quality criteria. In addition, the major pre- and post-analytical supporting systems will be described. Finally, a brief review about the current state of the automation of NAT testing will be given.

Pre-Analytical Supporting Systems

Olympus OLA2500

The Olympus OLA2500 (Olympus Medical Systems Europa GmbH, Hamburg, Germany) is an open sample management system for sorting, decapping, pooling and aliquoting of sample tubes. All tube types with a diameter of 10–17 mm and a height of 70–110 mm can be handled by the system. The OLA2500 is available in four different configurations, as sorter only or as sorting and aliquoting system each in a standard as well as in a high-speed version. The standard systems are capable of decapping, sorting and, where appropriate, archiving of up to 800 samples/h. The high-speed version can process up to 1,200 sample tubes/h.

The input area of the OLA2500 can be defined individually according to the needs of the user. Up to 300 sample tubes can be loaded at the same time in standard racks or directly in centrifugation buckets. Both primary and secondary as well as open and closed tubes can be processed simultaneously. The OLA2500 provides sample selective decapping based on user definable sorting rules. An integrated digital camera photographs each tube for automatic recognition of tube type and cap colour as well as for fill level detection and subsequent sample volume calculation. For short samples a priority list for processing can be created by the user.

The samples can be distributed by the OLA2500 system to a user definable number of secondary tubes or deep-well microplates without any carry-over. The respective microplates are assigned to each sample by positive bar code identification. The aliquoting is performed using special disposable tips which allow for pipetting volumes of 30–920 µl and which can be controlled by positive dispense monitoring. A stock of 420 tips can be stored on-board. Each secondary tube is automatically labelled with a bar code which contains all necessary information. The system is also able to perform sample pooling for NAT testing. Positive bar code identification then prevents mixing up of samples. Conductivity measuring during sample take-up as well as positive dispensing verification by weighing

of the pool tube guarantee an accurate pooling process. Short samples are automatically marked and put aside. Pressure transducers provide automated clot detection.

The Olympus OLA2500 is an open system which allows for direct sorting of sample tubes into the analyzer racks of all commonly used automated testing systems. The system provides flexibility concerning adaptability to future developments like new rack types.

The Olympus OLA2500 system is capable of a bi-directional exchange of information with the laboratory information management system (LIMS). Alternatively, Olympus offers a sorting drive software for the OLA2500. The system provides a complete documentation of the sample flow as well as all microplate and pool population data.

Sarstedt PVS

The Sarstedt PVS (Sarstedt AG & Co, Nümbrecht, Germany) is an open, individually configurable sample management system. The standard version includes sorting, decapping and recapping of sample tubes. Optionally additional modules for sample volume calculation as well as sample aliquoting into secondary tubes and deep-well plates can be integrated.

The input area of the Sarstedt PVS is equipped with a continuous loading function. All tubes with a diameter of 13–16 mm and a height of 75–100 mm can be handled routinely. For divergent tube sizes, the compatibility has to be checked. The samples can be sorted by bar code recognition, by cap colour identification or optionally by tube type recognition. Optionally, a special module for sample volume calculation can be integrated into the system. For correct performance of fill level detection and sample volume calculation, the tube must provide an unlabelled reading window with a width of 6 mm. Short samples can be recognised and put aside.

When no aliquoting of samples is performed, the Sarstedt PVS can process up to 1,400 samples/h. An optional aliquoting unit with a single aliquoting arm can easily be integrated; aliquoting of samples reduces the throughput to 400 samples/h. Up to 600 disposable tips with a volume of 1,000 µl can be stored on-board; the application of smaller disposable tips is also possible. A stock of 250 secondary tubes which are automatically bar code labelled can also be stored in the aliquoting unit. Direct aliquoting for archiving into deep-well plates is also possible; in this case, the microplates have to be scanned manually. The minimal aliquoting volume is 50 µl; the maximal volume is defined by the total volume of the respective sample. The aliquoting process is monitored by liquid level detection as well as detection of clots, foam and short samples.

A unique feature of the Sarstedt PVS is the capability of recapping of samples. For this purpose universal lamella plugs are used which can be decapped again by the system if necessary.

The open sample racking unit accepts all commonly used analyzer racks and can easily be adapted to new rack formats.

The Sarstedt PVS continuously exchanges data with the LIMS. The detailed processing protocol data can be transmit-

ted individually for each sample or in datasets for sample batches.

Tecan Genesis FE500™

The Tecan Genesis FE500 system (Tecan Deutschland GmbH, Crailsheim, Germany) is a CE-IVD compliant front-end automation workcell for the pre-analytical sample management. The system is capable of pre-sorting tubes, sample volume inspection and centrifugation. Decapping of tubes, aliquoting of samples, destination sorting and sample racking are integrated work steps of the Tecan Genesis FE500. The system is capable of processing up to 500 samples/h. Compared to other pre-analytical systems, the Tecan Genesis FE500 is very small-sized, but anyhow comprises all typical features of a front-end system.

The tube loading unit of the Tecan Genesis FE500 accommodates up to 80 tubes at one time and provides a continuous loading function. Glass and plastic tubes with a diameter of 11.5–16 mm and a height of 65–100 mm can be processed. In the tube loading unit a pre-sorting of samples is already performed in order to streamline the workflow.

The Tecan Genesis FE500 comprises a fully automated centrifuge which allows for the integration of this process into the pre-analytical workflow. Samples are automatically balanced as well as loaded and unloaded from the centrifuge which is placed below the deck. Centrifugation time, speed and temperature are user definable. This provides a flexible treatment of samples designated for special analyses. Samples not to be centrifuged bypass the centrifugation unit and are directly loaded onto the conveyor system.

The tube inspection unit is equipped with a special laser beam which penetrates up to three overlapping layers of different labels onto the tube for evaluating the tube contents. For optimised performance the tube can be rotated automatically to get access to the best reading window. In the tube inspection unit the identification of the separation layers (tube bottom, blood cake, gel, serum/plasma, and air interfaces) takes place. Basing on the acquired data, the available sample volume as well as the remaining volume for archiving is calculated. The maximum tip immersion depth is assessed to avoid touching of gel or blood cake during sample aspiration. The measured values are transmitted to the aliquoting unit as well as to the LIMS. Suspicious samples are identified and put aside.

The sample transport within the Tecan Genesis FE500 system is realised by a dual-lane conveyor system. This unit provides continuous monitoring of sample flow through sample bar code and carrier identification.

The decapping unit is able to handle standard as well as screw caps. For decapping, the tube is moved into a stainless steel casing to minimise contamination and infection hazards.

The Tecan Genesis FE500 system comprises a secondary tube supply with enough capacity for stocking up to 1,500 secondary tubes with a diameter of 13 mm and a height of 75 mm. A thermo-transfer printer with a stock of approximately 8,500

Table 1. Features of the main pre-analytical supporting systems

	Olympus OLA2500	Sarstedt PVS	Tecan Genesis FE500
Applicable tubes	diameter: 10–17 mm height: 70–110 mm	diameter: 13–16 mm height: 75–100 mm	diameter: 11.5–16 mm height: 65–100 mm
Sample throughput	800/1,200 samples/h (standard/high speed version)	1400/ca. 400 samples/h (without/with aliquoting)	500 samples/hour
Integrated centrifuge	not available	not available in standard version, optional integration possible	yes
Sample sorting	yes	yes	yes
Sample volume calculation	yes	yes	yes
Decapping	yes	yes	yes
Pooling	yes	not available	not available
Aliquoting	yes (30–920 µl)	yes (50 µl – total sample volume) (optionally)	yes (50–,3,000 µl)
Disposable tip supply	420 tips	600 tips (optionally)	288 tips
Monitoring of correct pipetting	yes	yes (optionally)	yes
Labelling of secondary tubes	yes	yes (optionally)	yes
Recapping	not available	yes	not available
Configurable sample racking unit	yes	yes	yes
Communication with LIMS	yes	yes	yes

labels is integrated into the secondary tube supply unit. Each secondary tube is labelled automatically with a user definable bar code and text label before being transferred to the aliquoting unit.

The aliquoting unit requires positive primary and secondary bar code identification. Aliquots of 50–3,000 µl can be produced according to the specific needs of the user. The aliquot arm uses disposable tips to avoid contaminations. The disposable tip supply contains up to 288 tips. During the aliquoting process, serum volume and clot detection is performed.

The completely configurable sample racking unit accepts various analyser racks from different manufacturers. The worktable of this unit can be divided into up to 31 different destination sectors.

The software is capable of a bi-directional exchange with the LIMS, and it provides real-time feedback on all modules. All important information is displayed on one screen. The software also includes a validation programme which ensures that the system performance meets the specifications. The process documentation meets the requirements of the IVD Directive. The Tecan Genesis FE500 system needs a controlled operating environment within a temperature range from 15–32 °C and a relative humidity from 30–80%. Air conditioning is strongly recommended by the manufacturer (table 1).

Automated Serological Testing Systems

The Abbott Systems: AxSYM®, PRISM® and ARCHITECT® i2000_{SR}

Abbott (Abbott GmbH & Co. KG, Wiesbaden, Germany) is offering three major systems suitable for blood banking facili-

ties in Germany: the AxSYM, the PRISM and the new ARCHITECT system. The Abbott systems are closed systems, i.e. only definite tests which are designed for the particular system can be processed.

AxSYM

The AxSYM system was introduced in 1994. It is applicable both in blood banking facilities and in clinical chemistry laboratories. The testing methods applied are microparticle immunoassay and fluorescence polarisation immunoassay. Up to 20 different test parameters can be processed simultaneously by the AxSYM system, including HIV Ag/Ab Combo, HBsAg, HBsAg confirmatory, anti-HBc, anti-HBc IgM, anti-HCV, anti-CMV IgG, anti-CMV IgM, anti-HAV IgG/IgM, anti-HAV IgM, HBeAg and anti-HBe. There is no syphilis test available which is compatible with the AxSYM system. The routine testing for HIV (Combo assay), HBsAg, anti-HBc and anti-HCV requires a total sample volume of 507 µl; additional testing for anti-CMV IgG consumes another 55 µl of sample. No malfunction of assay performance has yet been observed for samples which had been frozen before (like in look back procedures).

Primary tubes with a diameter of 12–16 mm and a height of 93–102 mm as well as secondary tubes with a diameter of 12–16 mm and a height of 64–76 mm can be processed. The AxSYM system can handle tubes with and without a gel layer. Serum as well as plasma samples can be applied. The sample racks cannot be centrifuged, but can be filled by various pre-analytical sorting systems. Direct pipetting of samples out of deep-well plates and other microplates is not possible. Samples are distributed by integrated needles with a carry-over rate of <0.1 ppm. The precision mechanics provide high accuracy of pipetting. The sample distribution process is monitored

by pressure difference measuring. The AxSYM system can be loaded with up to 60 primary tubes and offers a throughput of 60–120 tests/h. The walk-away system provides the possibility of continuous loading of samples, reagents and consumables. The ready-to-use reagents are stable for 336 h within the system.

The AxSYM system generates solid as well as liquid waste. The solid waste consists of one matrix cell and one reaction cartridge per sample. Furthermore, up to 640 ml liquid waste/h are produced by the system.

The software is capable of a bi-directional exchange with the LIMS. The AxSYM system provides local memory capacity for up to 1,500 test results as well as for 5,000 control results. The data can also be saved on diskette.

The AxSYM is a medium-sized testing system which shows successful operation within a temperature range of 17–30 °C and a relative humidity of 15–85%.

The operator convenience as well as the reliability of the AxSYM system have been improved in the latest configuration, AxSYM Plus.

PRISM

The PRISM system provides GMP compliant complete automation of serological testing for infectious diseases. It has been designed to meet the special needs of a high throughput blood banking facility. The ChemiFlex® (Abbott GmbH & Co. KG) testing technology used in the assays is a highly sensitive combination of microparticles as solid phase and chemiluminescence measurement as detection system. The novel technology provides assays with very high sensitivity and specificity. The PRISM system is equipped with six independent channels (including one spare channel) for the testing for anti-HCV, HIV Ag/Ab Combo, HBsAg and anti-HBc. Moreover, an HBsAg confirmatory assay and testing for anti-HTLV I/II can be performed. Currently, additional tests for Chagas' disease and malaria as well as a HCV Ag/Ab Combo assay are being developed. For the PRISM system, no assays for the detection of syphilis or CMV infection are available. The routine testing for HIV (Combo assay), HBsAg, anti-HBc and anti-HCV requires a total sample volume of 350 µl. The PRISM assays show regular performance with samples which had been frozen before (like in look back procedures).

Samples are distributed using disposable tips to avoid contaminations. Serum as well as plasma samples can be applied. The PRISM system is capable of processing various tube types, including tubes with a gel barrier. The sample racks cannot be centrifuged but can be filled by various pre-analytical sorting systems. Direct pipetting of samples out of deep-well plates and other microplates is not possible. The PRISM system provides high sample throughput (160 samples/h corresponding to 800 tests/h). The first result is available after 54 min, continuously followed by another result every 40 s. Up to 280 samples can be loaded simultaneously. Moreover, the walk-away system offers continuous sample processing, processing of

emergency samples in less than 1 h and software controlled repeated testing. The reagents can be stored in the on-board refrigerator which provides enough capacity for reagents required for 5,000 tests per assay. The functionality is monitored at each processing step; and the performance of reagents and system is controlled throughout the process. The monitoring system includes positive sample identification during the whole process, monitoring of sample and reagent uptake as well as dispensing, monitoring of the pump function, necessity of valid controls for the release of results, and validation of critical functions.

The PRISM system generates solid waste in the form of one reaction plate per 16 samples. No liquid waste is produced by the system.

The software of the PRISM system provides complete electronic documentation of test, user, date and time, lot number and shelf life, information about controls, calibrators and their status, sample IDs and results, error codes and brief description as well as the request for system maintenance. Moreover, the software is capable of a bi-directional exchange with the LIMS. The PRISM system provides local memory capacity for up to 100 test batches. The data can also be saved on diskette; possibilities for data storage on CD or USB are currently being developed.

The PRISM is a large-sized, high throughput testing system which shows successful operation within a temperature range of 15–30 °C and a relative humidity of 20–80%.

ARCHITECT i2000_{SR}

The ARCHITECT i2000_{SR} system also is a GMP compliant, completely automated system for serological testing for infectious diseases. The ChemiFlex technology already described above has been used for the development of state-of-the-art assay reagents with excellent sensitivity and specificity. All serological tests necessary for the screening of blood donors (anti-HCV, HIV Ag/Ab Combo, HBs Ag, HBsAg confirmation, anti-HBc, anti-HBc IgM, anti-HBs, HBeAg, anti-HBe, anti-HAV IgG, anti-HAV IgM, anti-CMV IgG, anti-CMV IgM and syphilis) can be performed by the ARCHITECT i2000_{SR} system. Further tests are currently being developed, e.g. HCV Ag, HCV Ag/Ab Combo, CMV avidity, toxoplasmosis avidity and anti-HTLV I/II. The routine testing for HIV (Combo assay), HBsAg, anti-HBc, anti-HCV and syphilis requires a total sample volume of 250 µl; additional testing for anti-CMV IgG requires another 25 µl of sample. The ARCHITECT assays show regular performance with samples which had been frozen before (like in look back procedures).

Nearly all tube types are compatible with the ARCHITECT i2000_{SR} system, any tubes with a diameter of 10–16 mm and a height of 75–100 mm can be used. The sample racks of the ARCHITECT i2000_{SR} system can directly be centrifuged. Distribution of samples out of deep-well plates and other microplates is not possible. Samples are distributed by integrated needles with a carry-over rate of <0.1 ppm.

One-step assays and two-step assays are processed within the same time (200 tests/h). The first result is already available after 28 min. The system is equipped with a novel, 3D robotic sample handler which increases productivity by allowing for permanent access to the samples as well as random sample loading and by providing special emergency positions. Emergency samples can be processed by an additional pipetting arm. The system also allows for an automated re-run of samples. The ARCHITECT *i2000*_{SR} system provides 25 cooled reagent positions for up to 12,500 tests, the colour coded reagents as well as the calibration excel in an on-board stability of 30 days. The reagents are labelled with a 2D bar code which provides information concerning assay, lot number, kit size, shelf life and calibration curve. There are two kit sizes available, for 100 and 500 tests. The extensive stock of consumables which can be reloaded at any time permits a walk-away time of 5 h. The reagent buffer is continuously reloaded by the system. The in-process monitoring includes not only positive identification of samples and reagents but also pressure monitoring during pipetting of samples.

Up to 1,000 reaction tubes can be collected in the integrated waste container. In addition, the ARCHITECT *i2000*_{SR} system produces about 5.5 l liquid waste which can be disposed into the sink.

The user-friendly software provides access control, complete electronic documentation and a special quality control package. Furthermore, the software is capable of a bi-directional exchange with the LIMS. The ARCHITECT *i2000*_{SR} system provides local memory capacity for up to 35,000 test results as well as for 50,000 control results. The data can also be saved on CD.

The ARCHITECT *i2000*_{SR} is a medium-sized testing system which shows successful operation within a temperature range of 15–30 °C and a relative humidity of 10–85%.

For facilities with a low number of samples to be tested, a smaller-sized version of the ARCHITECT system, the ARCHITECT *i1000*_{SR}, will soon be available. This new system will provide a throughput of up to 100 tests/h with an identical range of different tests.

Bio-Rad ELITE™

The CE-IVD and GMP compliant, FDA approved Bio-Rad ELITE system (Bio-Rad Laboratories GmbH, Munich, Germany) was developed on the basis of the Hamilton MICRO-LAB STARFAME system consisting of the sampler STAR and the processor FAME. Both elements can be used independently and can be combined according to the individual needs (e.g. 2 samplers and 1 processor). The ELITE system is not yet established in Germany, but there are systems already installed in Finland, Portugal and France.

Various microplate assays can be processed by the ELITE system including the MonoLisa® HCV Ag-Ab Ultra (Bio-Rad

Laboratories GmbH) which currently is the worldwide only HCV Combo test available. This test excels in a very high sensitivity and specificity and yields positive results already 4–5 days after positive results in NAT testing. Further assays yet validated for the ELITE system are the MonoLisa HBsAg Ultra assay which provides full mutant detection, the MonoLisa Anti-HBc Plus assay, the Genscreen Ultra HIV Ag-Ab Combo assay, the Syphilis TA EIA II, the Behring CMV Total Ab assay or the Bio-Kit CMV Total Ab as well as different HTLV I/II assays. Moreover, archiving of samples by dispensing into deep-well plates can be performed by the system.

The sampler comprises an 8-channel pipettor arm with independently moveable needles. The disposable tips are fixed by a special hooking mechanism essential for the continuous pressure monitoring which allows for liquid level detection as well as for detections of clots, foam and short samples. The correct performance of the individual pipetting steps can be controlled by the deposition proof monitoring system basing on the photometric measuring of the colour shift after each pipetting step. Up to four different tests per sample can be performed simultaneously on up to three different microplates. Samples including controls and conjugate are distributed in 14, 18 or 21 min when one, two or three different tests are performed respectively. Up to 928 samples on 29 racks à 32 positions can be processed simultaneously due to the continuous loading system and the autoloader tray. Up to 960 disposable tips can be stocked on-board. Depending on the number of different assays, 450–820 tests/h can be performed. Sample tubes, reagents, and microplates are identified by an internal mobile bar code reader, whereas the washing solutions have to be scanned manually. The reagent dispensers each comprise a disposable syringe which is stored in the corresponding reagent container. The syringe has a total volume of 20 ml, whereas the reagent container itself contains a total volume of 100 ml. The respective dispense volume of 20–200 µl per well is dispensed in 5 µl aliquots. The dispensing process includes capacitive liquid level detection. In the wash solution stack four containers à 3 l for each washer can be stored. The fill level of the containers is controlled by capacitive liquid level detection. The wash and rinse solution containers can be exchanged during operation.

The ELITE system is available in two different configurations: for large facilities with high sample numbers the system can be equipped with two washer modules each of them consisting of three rows à 8 needles, 24 reagent positions and 20 incubation positions, whereas for smaller facilities the system can be fit out with 1 washer module, 16 reagent positions and 20 incubation positions. Several reagent positions can be used for stocking the same reagent. The washer module provides capacitive liquid level detection for each needle. The reader photometer is equipped with 8 measuring channels as well as 1 additional reference channel so that 8 wells can be measured simultaneously. The reader can be equipped with up to 8 optical filters with wavelengths from 340–750 nm. The transport of the mi-

Table 2. Throughput of the OCD system depending on system configuration and number of different tests

ML STAR IVD configuration	ML FAME configuration	Throughput per 7-hour shift
8 channels	16/20	one test per sample: 1,980 samples
12 channels	24/20	one test per sample: 2,790 samples
12 channels	16/20	four tests per sample: 450 samples (1,800 tests)
12 channels	24/20	four tests per sample: 540 samples (2,160 tests)

croplates to the different modules is coupled to the pipettor arm.

The software of the ELITE system, Aurora Gold, is capable of communicating with different systems and LIMS, comprises an integrated archive database as well as a host software interface. The ELITE system can be combined with the Olympus OLA system for sample sorting and with the smaller Bio-Rad EvolisTM system both for further tests and as back-up system.

Dade Behring Quadriga BeFreeTM

The Dade Behring Quadriga BeFree system (Dade Behring Marburg GmbH, Marburg, Germany) consists of one liquid handling platform combined with optionally one or two BEP[®] III analysers (Dade Behring Marburg GmbH). The open system can process any microplate based ELISA. Amongst others, the Quadriga BeFree system is compliant with various Dade Behring tests for the serological screening of blood donors for infectious diseases. For example, the Enzygnost[®] HIV Integral II test consists of both an antibody sandwich assay for detection of the HIV p24 antigen and an antigen sandwich assay for detection of anti-HIV 1/2/O IgG and IgM. According to the manufacturer, this HIV Combo assay shows high seroconversion sensitivity, yielding a positive result with a specificity of 99.89% up to 7 days earlier than other Combo tests. The Enzygnost HBsAg 5.0 assay excels in the capability of detecting all HBsAg mutants known so far. It also shows high sensitivity, including seroconversion sensitivity, as well as high specificity. Further well established Dade Behring tests are the Enzygnost Anti-HBc monoclonal assay, the Enzygnost Syphilis assay, the Novagnost[®] Parvovirus B19 IgG assay and the Enzygnost Anti-CMV/IgG+IgM assay. The routine testing for anti-HIV, HBsAg, anti-HBc, anti-HCV and syphilis requires a total sample volume of 270 µl; additional testing for anti-CMV consumes another 10 µl of the sample. No malfunction of assay performance has yet been observed for samples which had been frozen before (like in look back procedures). Samples and controls are distributed using Teflon[®]-coated steel needles which are decontaminated after each pipetting cycle to minimise contaminations. The samples are distributed by multi-pipetting into the different tests. Shortly after distribution, the samples are released in batches of 88 samples and are available for other tests. Due to the use of steel needles contaminations cannot completely be excluded. The carry-over risk for subsequent NAT testing of the sample has not yet

been examined. The reagents are distributed using plastic syringes to eliminate dead volume and possible contaminations. The Quadriga BeFree system is capable of processing all standard tubes with a diameter of 11–16 mm, independent of the manufacturer. Special formats like false bottom tubes may also be employed. The sample tubes can be placed into the Quadriga BeFree strip racks by various established pre-analytical systems (e.g. Sarstedt BBS/PVS, Olympus OLA, etc.). Tube types are identified by means of the respective strip rack. The possibility of direct sample distribution out of deep-well plates is planned but has not yet been realised.

In less than 5 h, the Quadriga BeFree system can process 440 samples when 3 tests are performed, or 264 samples when 5 tests are performed. Up to 6 different assays per microplate can be interpreted by the system. Pipetting of samples into deep-well plates for archiving can also be performed by the system. Besides a self-test during initialisation, the monitoring system includes an integrated quality control of positive and negative controls and reagents as well as microplate identification. During the process, the system demands positive bar code identification of samples, reagents and microplates. Liquid level detection is applied at various process steps in order to detect air bubbles, clots and short samples. The correct sample transfer from the primary tube to the micro-well is verified by photometric reading. The liquid dispensing is controlled by monitoring of the stepper motor. All incubators are monitored regarding accurate temperature. The photometer is calibrated automatically to guarantee optimal light intensity, and each sample result is confirmed by a second reading.

In large part, only liquid waste is generated by the Quadriga BeFree system. The liquid handling platform comprises a waste container with a volume of 20 l. Each analyser is equipped with waste bottles with a volume of about 2 l. These waste containers are sufficient for the screening of more than 440 samples and accordingly 10 microplates per analysing system. The liquid waste can normally be disposed into the sink. The user-friendly software provides full process control, including donor and sample management, job list distribution, result collection and interpretation, consolidation of sample results for individual donors, long-term data storage, back-up function as well as complete documentation of test and validation protocols comprising the individual processing steps. The high flexibility in protocol programming enables the user to customise the application protocols. The software also comprises detailed operator guidance for the daily and weekly system maintenance.

Comparable to the Bio-Rad ELITE system, Ortho Clinical Diagnostics (OCD; Neckargemünd, Germany) uses the Hamilton MICROLAB STARFAME system as platform for automation of serological testing for infectious diseases. As already described this system consists of the pipettor Hamilton MICROLAB STAR IVD and the processor Hamilton MICROLAB FAME. The CE-IVD compliant OCD system is designed as an open system which can process any microplate based ELISA, independent of the manufacturer. Samples, reagents and microplates have to be loaded manually. The system can be configured to match the individual needs of the user concerning sample throughput. As high throughput version a 12-channel pipettor can be combined with a processor equipped with 24 reagent positions and 20 incubation positions. In this configuration, 2,700 tests per 7-hour shift can be performed. Alternatively, the OCD system can be fit out as a medium throughput version with an 8-channel pipettor and a processor equipped with 16 reagent positions and 20 incubation positions (table 2).

Up to 576 sample tubes in 18 sample carriers can be loaded simultaneously. The testing of 88 samples for anti-HIV, anti-HCV and HBsAg, including archiving of samples in additional microplates, is completed after 3 h, the testing of 176 samples after less than 4 h, the testing of 264 samples after about 4 h, the testing of 352 samples after 5 h, the testing of 440 samples after less than 6 h and the testing of 528 samples after less than 7 h. Additional testing of the samples for anti-CMV or testing for HBsAg, anti-HIV and syphilis can be performed in 15 min longer total time.

The OCD system provides various security and safety features, including access control via pass code and positive identification of sample tubes, reagents and microplates. Furthermore, a constant pressure monitoring is performed throughout the process, including liquid level detection as well as total aspiration and dispense monitoring. The individual points of the pressure tolerance curve can be adapted to the user's given factors. The software assures a high standard of process documentation and traceability of all process steps. The scheduling software manages the workflow during the entire day while still allowing for changes. The system maintenance is also software controlled (table 3).

Automation of NAT Testing

While automation of serological testing for infectious diseases is already well established and various automated systems have been realised, the automation of NAT testing is still in the stage of development. First approaches have been made by Chiron and Gen-Probe (now Novartis) with the PROCLEIX® TIGRIS® system which allows for fully automated NAT testing of individual samples and small pools of 16

donors for HIV-1, HCV, and HBV. As the pooling step is not integrated into the system, complete automation of the testing process is only provided for individual samples. The assays used in the PROCLEIX TIGRIS system are based on the transcription-mediated amplification (TMA) technology. This technology uses reverse transcriptase for creating a cDNA strand of the target nucleic acid. RNA polymerase is used for synthesising new RNA molecules from the cDNA. The TMA technology allows for synthesising up to 1,000 new RNA copies per cycle; in contrast to PCR the TMA reactions are performed at isothermal temperature. The PROCLEIX TIGRIS system requires controlled environmental conditions within a temperature range of 15–25 °C and a humidity of 20–85%.

Recently, another fully automated NAT testing system has been developed by Roche with the new cobas® s 201 (Roche Pharma AG, Grenzach-Wyhlen, Germany). This system provides automated virus extraction as well as automated NAT testing of individual donors and minipools of 6 donors for HIV-1 (groups M and O), HIV-2, HCV and HBV. NAT testing for these viruses is realised in one single multiplex real-time PCR. An additional multiplex PCR for the detection of HAV and parvovirus B19 is currently being developed. In the cobas s 201 system, the pooling step is performed by one or several Hamilton MICROLAB STAR pipettors equipped with a special pooling software. The routine suitability of the cobas s 201 system, however, remains to be assessed.

In the multiplex assays of both the PROCLEIX TIGRIS system and the cobas s 201 system probes labelled only with one dye are applied. Thus, in case of a positive test result, several discriminatory assays have to be performed to identify the infectious agent and to exclude possible contamination with a second virus. This discrimination procedure is cost- as well as labour-intensive and, moreover, time-consuming.

Besides these two manufacturers, there is a concerted project of several German blood donation services with the aim of developing a completely automated NAT testing system which meets exactly the needs of the users. This system which is currently being finalised is capable of extracting viral nucleic acids and subsequent real-time PCR testing of individual samples, and pools of up to 96 donors for HIV-1 (groups M, N and O), HIV-2, HCV, HBV, HAV and parvovirus B19. The pooling process is not directly integrated into the system but can be performed by various pre-analytical systems. The newly developed automated testing system provides identical sensitivity for individual and pooled samples. This is realised by centrifugation of pools for pelleting of viruses before extraction. A further increase of sensitivity for confirmatory testing can be reached by applying a greater sample volume. For multiplex assays different probe dyes are used for the detection of the individual viral nucleic acid sequences. In case of a positive result, the respective virus can be identified without the necessity of a discriminatory assay.

Table 3. Features of the described automated serological testing systems

	Abbott PRISM	Abbott AxSYM	Abbott ARCHITECT i2000SR	Bio-Rad ELITE	Dade Behring Quadriga BeFree	OCD STARFAME Combo
Open/closed system	closed	closed	closed	open	open	open
Available tests	HBsAg HBsAg confirmation anti-HBc anti-HCV HIV Ag/Ab Combo anti-HTLV-I/II <i>under development:</i> HCV Ag/Ab Combo Chagas' disease malaria	HBsAg HBsAg confirmation anti-HBc total anti-HBc IgM anti-HCV HIV Ag/Ab Combo syphilis (TPPA) anti-CMV IgG anti-CMV IgM anti-HAV IgG anti-HAV IgM anti-HAV IgM HBeAg anti-HBe <i>under development:</i> HCV Ag/Ab Combo HCV Ag CMV avidity toxoplasmosis avidity anti-HTLV I/II	HBsAg HBsAg confirmation anti-HBc total anti-HBc IgM anti-HCV HIV Ag/Ab Combo anti-CMV IgG anti-CMV IgM anti-HAV total anti-HAV IgM HBeAg anti-HBe	HBsAg anti-HBc HCV Ag/Ab Combo HIV Ag/Ab Combo Syphilis ELA anti-CMV total HTLV-I/-II	HBsAg HBsAg confirmation anti-HBc anti-HCV anti-HIV syphilis ELISA anti-CMV total parvovirus B 19 IgG	any microplate based ELISA can be processed
Testing method	chemiluminescence	microparticle immunoassay fluorescence polarisation immunoassay	chemiluminescence	microplate based ELISA	microplate based ELISA	microplate based ELISA
Applicable tubes	no information available	<i>primary tubes:</i> diameter: 12–6 mm height: 93–102 mm <i>secondary tubes:</i> diameter: 12–16 mm height: 64–76 mm	diameter: 10–16 mm height: 75–100 mm	no information available	diameter: 11–16 mm	no information available
Centrifugable sample racks	no	no	yes	no	no	no
Compatibility with front-end systems	yes	yes	yes	yes	yes	yes
Sample distribution	disposable tips	needles carry-over rate < 1 ppm	needles carry-over rate < 1 ppm	disposable tips	Teflon coated needles	disposable tips

Table 3 to be continued on the following page.

Table 3. Continuation

	Abbott PRISM	Abbott AxSYM	Abbott ARCHITECT i2000 _{SR}	Bio-Rad ELITE	Dade Behring Quadriga BeFree	OCD STARFAME Combo
Monitoring of correct pipetting	liquid level detection pressure difference measuring	pressure difference measuring	pressure difference measuring	liquid level detection detection of clots, foam, and short samples deposition proof monitoring	liquid level detection detection of clots, foam, and short samples deposition proof monitoring	liquid level detection detection of clots, foam, and short samples by total aspiration and dispense monitoring constant pressure monitoring
Sample throughput	160 samples/h corresponding to 800 tests/h	60–120 tests/h	200 tests/h	450–820 tests/h	up to 440 samples in 5 h, if 3 tests per sample are performed Up to 264 samples in 5 h, if 5 tests per sample are performed	up to 2,790 tests per 7-hour shift in staggered intervals exact number of tests depends on processor configuration and on the number of tests per sample
Waste	1 reaction plate per 16 samples	1 matrix cell and 1 reaction cartridge/sample 640 ml liquid waste/h	1 reaction cartridge/sample 5.5 l liquid waste/h	microplates liquid waste	microplates liquid waste	microplates liquid waste
Software	bi-directional exchange with LIMS local memory: 100 test batches data storage: diskette, storage on CD/USB under development complete electronic documentation of all process steps software controlled request for system maintenance	bi-directional exchange with LIMS local memory: 1,500 test results + 5,000 control results data storage: diskette	bi-directional exchange with LIMS local memory: 35,000 test results + 50,000 control results data storage: CD complete electronic documentation of all process steps quality control package	communication with LIMS and (pre-) analytical systems complete electronic documentation of all processes plate ore tube archive management	bi-directional exchange with LIMS long-term data storage including consolidation of results for individual donors complete electronic documentation of all processes customizable application protocols operator guidance for system maintenance	data exchange with LIMS complete electronic documentation of all processes software controlled maintenance and verification

Conclusions

When speaking about automation in serological and NAT testing of blood donors for infectious diseases, many factors have to be considered.

As blood donation services are considered as pharmaceutical enterprises in the current legal situation, they have to fulfil strict legal restraints concerning GMP compliance. These include standardisation of processes to a great extent, minimisation of influencing factors including human errors, extensive documentation as well as traceability of samples and the respective data over a long period. All these factors are provided by automated testing systems.

Automated testing systems help to minimise the infection risk for the staff. Though correct sample handling during manual work prevents infection with many pathogens, there always remains a residual risk. Moreover, considering the constant emergence of new pathogens which might be transmitted by aerosols, infections of the personnel during handling of samples can never completely be prevented.

Laboratory automation also contributes to the optimisation of the routine workflow. Many time-consuming and laborious manual activities, like decapping, pooling, aliquoting or archiving of samples, can easily be carried out by an automated system in only a short period of time. Incubation steps, which regularly also lead to substantial loss of working time – as they all the same tie the technician up to the respective assay – do not play a role when the test is performed by an automated system. Moreover, typical human failures which can lead to false test results, like mixing up of samples, pipetting errors or impreciseness as well as sample carry-over, are all but impossible in an automated system. Thus, the safety of the blood products can further be improved by automated testing when at least identical test sensitivity as that of the respective manual tests is presumed.

With regard to inspections by controlling authorities, the transparency of the testing process is improved. A completely controllable and transparent workflow is also an essential prerequisite for successful accreditation and certification of laboratories.

The available automated testing systems normally are equipped with prepared interfaces suitable for the connection to various LIMS. Vice versa, the common LIMS are also laid out for connection to automated systems of different established manufacturers. Thus, the technical integration of one or several automated systems can easily be accomplished.

More important, however, is the expedient integration of automated systems into the routine workflow of the respective laboratory. The number of samples which have to be tested within a certain time frame plays a decisive role for making the choice for an appropriate system. Furthermore, the required space and the availability of air conditioning which is necessary for the correct function of some automated systems have to be considered. Logistics of a reasonable sample flow have to be

taken into consideration and organised in advance. These include the possible integration of a pre-analytical system into the workflow. Besides decapping and sorting of tubes, the supporting systems are often capable of sample aliquoting. This feature can be used for generating aliquots for follow-up examinations. In this context, any carry-over risk has to be excluded. Furthermore, the traceability of the sample must be guaranteed. Thus, availability of respective safety features of the automated system has to be evaluated. These include the use of disposable tips, the prevention of aerosol formation and carry-over as well as correct labelling of secondary tubes or deep-well plates and traceability of the sample.

Concerning the diversity of automated systems for serological testing for infectious diseases, the user has to make a basic decision for an open or a closed system. Both system types have specific advantages and disadvantages. Closed systems are already optimised and validated with regard to particular assays. Therefore, they can be put into operation without further effort on the side of the user. On the other hand, the user does not have the opportunity to apply tests from other manufacturers which might show better sensitivity and/or specificity. Closed systems may be limited to a smaller palette of different assays, and they show only limited adaptability to new testing methods. In contrast, open systems provide high flexibility and adaptability. The manufacturers of the established open systems usually offer a panel of different assays for which the compatibility with the automated system has already been validated. To a large extent, the user can decide freely which assay from which manufacturer he wants to apply. In this case, however, assay and system performance have to be validated and documented according to the legal requirements. For that reason, real openness of such systems is only given if in parallel several automated assays for the detection of the same infection parameter are validated and approved by the inspecting authorities.

Perspectives

Currently, a distinct trend towards laboratory automation can be observed. The need for GMP compliance is one of the major propulsive factors. Another important advantage of automated systems is the optimisation of the relation of costs and performance. With regard to testing of blood donors for infectious diseases, automation of serological testing has already been established in many blood donation facilities. Due to the great variety of systems, it is possible to meet almost exactly the needs of any of the different-sized blood donation services.

By contrast, automation of NAT testing is still in its infancy. At the moment, only two fully automated NAT testing systems are available, a third one is currently being finalised. For small blood donation facilities, the installation of one of the available automated NAT testing systems is currently too ex-

pensive and, moreover, has to be regarded as over-dimensioned.

In this context, the question of necessity of general NAT testing should be mentioned. The recent Combo assays excel through very high sensitivity and specificity which almost equal NAT testing even in the very early stage of infection. Moreover, the costs of Combo assays are currently considerably lower than NAT tests. In Germany, NAT testing will be performed also in the future in order to guarantee the best possible safety of blood products. In other countries, however, the Combo assays might be considered as a possible alternative to NAT.

At the moment, the serological and the NAT testing is performed in strictly separated laboratories. This might change in the foreseeable future. First approaches concerning the combination of multiple automated supporting and serological testing systems to laboratory assembly lines are currently made, for example by OCD and Abbott. The additional integration of fully automated NAT testing systems remains just a question of time.

The developments in laboratory automation to be due for the highly specialised blood banking sector may lead to a monopolisation of manufacturers. In this regard, negative consequences like increasing costs, decreasing quality of assays and systems, decreasing reliability of manufacturers, deterioration of customer service, etc. have to be prevented as this could lead to a decrease in the safety of blood products.

Note

This review does not claim completeness. The presented automated systems are to be taken as examples, especially as not all addressed manufacturers have provided us with the necessary information about their systems.

Acknowledgements

We thank the manufacturers of the described automated systems for providing us with the necessary information.