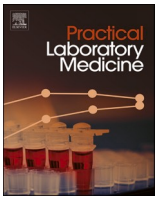
Practical Laboratory Medicine 39 (2024) e00364

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm



Evaluation and experience from routine use of chemiluminescence assays for serological screening of blood and plasma donations on the Alinity s system and the Alinity i system, two new fully-automated immunoassay systems in Poland

Aneta Kopacz [a,*], Dorota Kubicka-Russel [a], Grzegorz Liszewski [a], Alicja Bukowska [b], Sylwia Samek [c], Dorota Malka [d], Magdalena Łętowska [a], Piotr Grabarczyk [a]

[a] Institute of Hematology and Transfusion Medicine (IHTM), Department of Virology, Warsaw, Poland
[b] Regional Blood Transfusion Center, Poznań, Poland
[c] Regional Blood Transfusion Center, Kielce, Poland
[d] Regional Blood Transfusion Center, Warsaw, Poland

ARTICLE INFO

Keywords:
Blood donor screening
Alinity system
Chemiluminescence
Performance evaluation

ABSTRACT

In Poland, independent evaluations under the auspices of the Institute of Hematology and Transfusion Medicine (IHTM) are mandated for any new device, assay, systems for screening samples from whole blood and plasma donors prior to implementation by Blood Transfusion Center (BTC). In last 5 years, two new systems were introduced to the market by Abbott GmbH, namely the Alinity s and the Alinity i. The evaluations performed for these two systems included the assessment of sensitivity, specificity and precision for each of the four mandatory serological screening markers in Poland: Hepatitis B Surface Antigen (HBsAg), Hepatitis C virus antibodies (Anti-HCV), HIV antibodies (anti-HIV) and Syphilis antibodies (anti-*Treponema pallidum,* anti-TP). Sensitivity was assessed by testing seroconversion panels, HBsAg international reference standard, well characterized local samples, and dilution panels. Specificity was assessed by testing routine donor samples. The results from Alinity i assays were compared to the results from Abbott ARCHITECT i2000SR and Ortho VITROS 3600 assays, while the results from Alinity s assays were compared to the results of ARCHITECT i2000SR assays. The evaluation of the Alinity s and Alinity i assays for sensitivity (100 %), specificity (99,92–100 %) and precision generated results that were as good as or better than generated by routinely used systems, were within acceptance criteria, and met all requirements for screening blood donor samples in accordance with Polish regulations. The specificity of the assays in routine use by BTCs, analyzed after approximately 150,000 donations on both systems, was comparable to the specificity observed during the evaluations at IHTM.

* Corresponding author. Department of Virology, Institute of Hematology and Transfusion Medicine, 14 Indiry Gandhi Str, 02-776, Warsaw, Poland.

E-mail addresses: akopacz@ihit.waw.pl (A. Kopacz), drussel@ihit.waw.pl (D. Kubicka-Russel), gliszewski@ihit.waw.pl (G. Liszewski), alicja.bukowska@rckik.poznan.pl (A. Bukowska), wirusy@rckik-kielce.com.pl (S. Samek), d.malka@ihit.waw.pl (D. Malka), mletowska@ihit.waw.pl (M. Łętowska), pgrabarczyk@ihit.waw.pl (P. Grabarczyk).

https://doi.org/10.1016/j.plabm.2024.e00364
Received 13 May 2022; Received in revised form 18 December 2023; Accepted 18 January 2024
Available online 24 January 2024

1. Introduction

Safe blood play an important role in healthcare to save the lives of patients and as a basis for plasma-derived pharmaceutical products such as immunoglobulins and albumin. Self-sufficiency in obtaining plasma from European donors for the latter is a common aim of many European countries. The World Health Organization (WHO) published recommendations for screening of donated blood for transfusion-transmissible infections [1]. As part of the WHO publication, assurance the quality and continuity of test assays, and other consumables required for the screening of donated blood is recommended. In Poland, a Notice of the Ministry of Health (MOH) is in place, and it describes the requirements for collecting blood and its components, testing and preparation, storage, issuing, and transport for organizational units of the public blood service [2]. In chapter 10.14.1, the notice sets qualification specifications for newly introduced equipment that will be assessed by the Institute of Hematology and Transfusion Medicine (IHTM; http://www.ihit.waw.pl/). IHTM is the Polish reference center for these disciplines, acts as an advisory body to the MOH and taking over executive functions for the MOH as well. As such, IHTM is also one of the organizational entities of the public blood service in Poland performing substantive supervision over their activities. The Department of Virology of the IHTM is responsible for assessing all test systems and assays used for screening whole blood and plasma donations in Blood Transfusion Centers (BTC) in Poland. The aim of assessment is to assure that only the high-quality systems and assays in alignment with requirements of the "Notice" of the MOH and general GMP standards are applied. Thus, when new systems and assays become available, the IHTM is performing the assessment (either in-house or in collaboration with a BTC) like the newly developed Alinity s [3] and Alinity i [4] systems and its corresponding assays: HBsAg, HIV Ag/Ab Combo, Anti-HCV and Syphilis.

2. Materials and methods

2.1. Organization, systems and assays (details of experiments presented in Supplementary Table 1)

Evaluation of the Alinity s system in the laboratory of BTC in Warsaw (W) and evaluation of Alinity i in the laboratories in BTC in Poznań (P), BTC in Kielce (K) and in IHTM were complete during pre-approved protocols prepared by IHTM. Results from all evaluations were sent to IHTM for analysis and assessment.

Devices used:2 Alinity s systems at the BTC in Warsaw and 5 Alinity i systems (2 at BTC Poznań, 2 at BTC Kielce, and 1 at IHTM site). Results of the Alinity systems in BTC Warsaw, BTC Poznań and IHTM were compared to those generated by ARCHITECT i2000SR (Abbott Diagnostics, here ARCHITECT) and in BTC Kielce to VITROS 3600 (Ortho Clinical Diagnostics, here VITROS) systems [5]. In Alinity and ARCHITECT systems assays for detection HBsAg, anti-HCV Ab, HIV Ag/Ab Combo and anti-TP were used. In VITROS System assays for detection HBsAg, anti-HCV, anti-HIV and anti-TP were used.

2.2. Sensitivity

For the sensitivity assessment, we tested one seroconversion panel per assay, the WHO HBsAg IS and archived polish confirmed seropositive samples (repeat reactive confirmed in NAT, neutralization or Western Blot). The following seroconversion panels were tested: PHV 915 HCV, PHM 937 HBV, PRB 968 HIV and PSS 901 Syphilis (all SeraCare, Milford, MA 01757, USA). Dilution of the WHO 3rd IS HBsAg, NIBSC code: 12/226; (National Institute for Biological Standards and Control, Hertfordshire, EN6 3QG, UK) was tested for the HBsAg analytical sensitivity determination. Not all seroconversion panels and international standards were tested on all systems due to sample volume limitations. Five confirmed positive samples from the archives of the participating BTCs and another 15 samples from the IHTM were tested for each assay, HBsAg, Anti-HCV and HIV Ag/Ab Combo, while 10 samples were tested for Syphilis. Moreover, dilution panels prepared by the IHTM (consist of 6 positive and 1 negative sample) were tested in each laboratory. Additionally, archived seropositive samples: 14 HBsAg, 23 anti-HIV, 3 anti-TP and originally NAT yields: 14 HIV, 92 HCV, 23 HBV window period (WP) samples and 76 occult HBV were tested on Alinity i system at IHTM.

2.3. Precision and specificity

Panels prepared by each laboratory consisting of a negative, positive with low and high S/CO (Signal/Cut Off) value sample were tested on 2 Alinity s systems and 4 Alinity i systems. The testing was done in replicates for repeatability of 3/day for each sample, and for reproducibility of 1 for each sample in 3 consecutive days.

During the specificity evaluation, a total of 2,477 different specimens (2,176 serum and 301 plasma) from random blood donors collected at BTC Warsaw, Poznań and Kielce were tested. The data obtained during the initial assessment were compared to those of 176,670 samples tested on 2 Alinity s systems and 150,958 serum blood donor samples on 4 Alinity i systems.

Chi2 test (Statistica version 13, Palo Alto, CA, USA) was used to compare assays specificity during evaluation and in use testing. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Sensitivity

3.1.1. Seroconversion panels

All used in the study HIV and HCV assays, evaluated and reference, detected the same samples from seroconversion panels as positive and negative respectively. The HBsAg PHM 937 panel member 2 is a borderline sample with S/CO results near the cut-off and had elevated S/CO levels slightly below the cut-off in Alinity s and Alinity i HBsAg, with one result slightly above cut-off on the "K1" Alinity i system. No such elevation in S/CO values was observed in the VITROS HBsAg assay. The difference between the bleed dates of panel members 2 and 3 is 7 days. The Syphilis PSS 901 panel member 6 results were non-reactive in the VITROS Syphilis assay but reactive with the Alinity s and Alinity i Syphilis assays. The bleeding dates for this sample and the next one are 3 days apart (Fig. 1).

3.1.2. Analytical sensitivity - International standard (IS)

The WHO IS was used at a concentration of 0.13 IU/ml - far above the analytical sensitivities as claimed for the Alinity s HBsAg

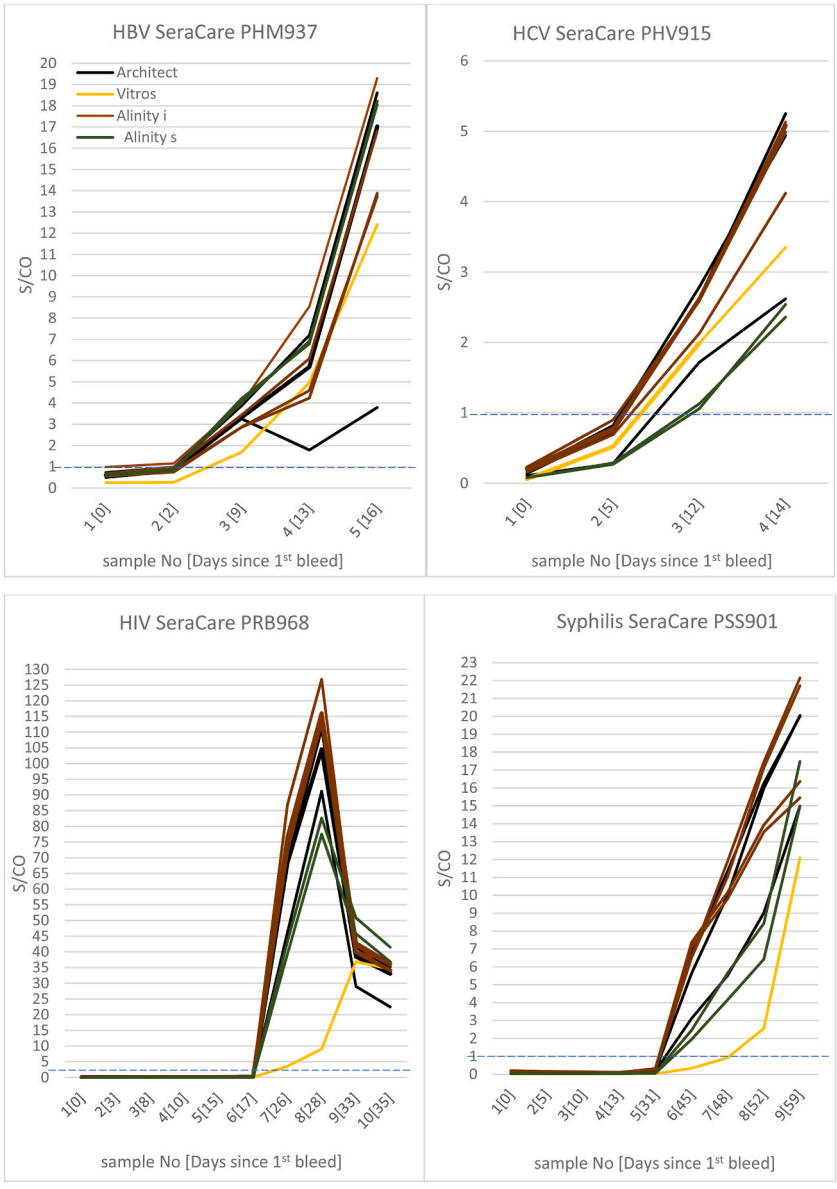


Fig. 1. Clinical sensitivity of seroconversion panels when tested on the Alinity i, Alinity s, ARCHITECT and VITROS assays (reactive S/CO > 1). S/CO – Signal/Cut Off.

assay (0.015–0.016 IU/ml determined by the 3rd WHO IS HBsAg, Instruction for use X.2017) and Alinity i HBsAg assay (0.020–0.021 IU/ml determined by the 2nd WHO IS HBsAg, Instruction for use III.2017). HBsAg IS Reactive results well above the cut-off were generated, as well as with the assays measured for comparison: Alinity i 4.80–5.78 S/CO Alinity s 6.30–7.16 S/CO, ARCHITECT 5.62–7.38 S/CO and VITROS 1.70–1.84 S/CO.

Fig. 2. Sensitivity of Alinity s and Alinity i systems in diluted Polish positive samples from blood donors with genotypes most often identified in Poland.

3.1.3. Clinical sensitivity - Confirmed positive samples from donors

Twenty positive samples (5 each for HBsAg, HIV Ag/Ab Combo, Anti-HCV, and Syphilis) from the respective BTCs were tested on each of the Alinity s and Alinity i systems and in parallel on ARCHITECT or VITROS systems at the centers. All samples were found reactive by the systems under evaluation and by those used for routine testing.

Fifty-five known positive samples from IHTM (15 each for HBsAg, HIV Ag/Ab Combo, Anti-HCV and 10 for Syphilis) were tested on Alinity s in "W", Alinity i in "P" and Alinity i in "K", as well as on the ARCHITECT and VITROS systems at the BTC centers. All samples on the Alinity s, Alinity i and ARCHITECT systems were found to be reactive, except 1 sample from the anti-HCV panel, which presented an elevated, but negative result on the VITROS assay (0.82 S/CO). All additional seropositive samples tested in IHTM were reactive on Alinity i system.

Out of NAT-yields 1 occult HBV (1.3 %), 4 WP HBV (17.4 %) and 1 WP HIV (7.2 %) were repeat reactive on Alinity i system (Supplementary: Table 2, 4, 5, 6).

Dilution panel: all positive HBsAg, Anti-HIV, Anti-HCV, Anti-Syphilis and negative dilution panel members in each evaluated and reference system were found respectively reactive and non-reactive (Fig. 2, samples characteristic Supplemental Table 7).

3.2. Precision

For assessment of precision, specific for BTC and Poland sets of panels consisting each of 1 negative, 1 low positive and 1 high positive sample were tested on both Alinity s and each of the Alinity i systems at each BTC site and IHTM. The results on all Alinity systems for all panels showed imprecision below 16 % for all positive panel members and for the large majority even below 5 % overall. (Supplementary: Table 3).

3.3. Specificity

Alinity s: 1,100 routine samples (1,000 serum and 100 plasma) were tested in parallel on the Alinity s (1 or both systems) and ARCHITECT. On Alinity s, all except 1 serum sample for HBsAg were non-reactive. The sample was also reactive in the ARCHITECT, but not confirmed on both systems with the corresponding HBsAg neutralization assays and was HBV DNA negative. Finally, this sample was considered as false-reactive. Two other serum samples were HBsAg repeat reactive in the ARCHITECT assay only but not confirmed by neutralization and DNA HBV. The specificity for the 4 Alinity s assays is shown in Table 1.

Alinity i: 1,377 routine samples (500 and 676 serum samples as well as 100 and 101 plasma samples respectively from BTC in Poznań and Kielce) were tested in parallel on Alinity i and on ARCHITECT (Poznań) or VITROS (Kielce). All samples gave non-reactive results on Alinity i except 1 sample for Anti-HCV, and 2 samples for Syphilis (both "P"). All three samples were confirmed positive in supplementary tests and classified as true-positives. The specificity of the 4 Alinity i assays is shown in Table 1.

4. Discussion

For screening samples from whole blood and plasmapheresis donors, it is important to use highly sensitive and specific tests. In Poland, it is the responsibility of IHTM to ensure that analyzers and assays comply with the set standards, prior to implementation to RBTCs, to avoid the use of products of minor quality [2]. Highly sensitive assays are needed to detect all positive samples, while highly specific assays are needed to avoid unnecessary donor deferrals and may cause costs for confirmation testing due to non-specific binding. The newly developed Alinity s and Alinity i systems with assays (HBsAg, HIV Ag/Ab Combo, Anti-HCV, Syphilis) in Poland underwent extensive testing to ensure that have high sensitivity and specificity standards and meet standards for good manufacturing practice (GMP). The Alinity s and Alinity i systems passed all requirements regarding sensitivity. All confirmed seropositive domestic samples were reactive with the new method. Testing of seroconversion panels, the HBsAg WHO IS and dilution

Table 1
Specificity (%) with confidence interval (%-%) and number of negative by the Alinity systems/total negative samples received during validation and in-use studies.

System	Study	HBsAg	HIV Ag/Ab	Anti-HCV	Anti-Syphilis
Alinity s	Validation	**99.91 %**	**100 %**	**100 %**	**100 %**
		99.49 %–100 %	99.67 %–100 %	99.67 %–100 %	99.67 %–100 %
		1,099/1,100	1,100/1,100	1,100/1,100	1,100/1,100
	In-use	**99.99 %**	**99.98 %**	**99.98 %**	**99.96 %**
		99.99 %–99.99 %	99.97 %–99.99 %	99.97 %–99.98 %	99.95 %–99.97 %
		176,655/176,670	176,636/176,670	176,630/176,670	176,606/176,670
Alinity i	Validation	**100 %**	**100 %**	**100 %**	**100 %**
		99.73 %–100 %	99.73 %–100 %	99.73 %–100 %	99.73 %–100 %
		1,377/1,377	1,377/1,377	1,376/1,376	1,375/1,375
	In-use	**99.97 %**	**99.98 %**	**99.91 %**	**99.96 %**
		99.96 %–99.98 %	99.97 %–99.98 %	99.89 %–99.92 %	99.95 %–99.97 %
		150,912/150,958	150,921/150,958	150,819/150,958	150,896/150,958

There were no statistical differences between specificity from validation and in-use study for all assays.
($p > 0,05$ in chi2 test).

panels for all 4 required markers have proven that the sensitivity of the Alinity assays is similar to or better than current methods in use. From tested NAT yields in Alinity i: 1,3 % occult HBV infection, 17.4 % HBV window period (17.4 %) and 7.1 % HIV WP samples were detected, which show that Alinity HBsAg and HIV Ag/Ab Combo test is more sensitive than the assays used in the past ("Supplementary" Table 2). The results from HBV seroconversion panel testing indicate higher sensitivity of Alinity i as well. Alinity i HBsAg assay detected one bleed that was negative by the VITROS assay (Fig. 1). During the evaluation the Alinity s and Alinity i systems showed high specificity (100 % and 99.92–100 %), comparable to the routinely used ARCHITECT assay (99.91–100 %). After testing about 150,000 blood donor samples in routine use of Alinity s and Alinity i systems the specificity did not differ from that observed in the evaluation (Table 1). The specificity of Alinity s in Poland is comparable to the specificity observed during evaluations performed in other European countries (99,88–100 %) [6–11]. The data from these studies indicate that new Alinity systems present comparable specificity to systems such as ARCHITECT, VITROS, PRISM and Enzygnost DiaSorin. High specificity is desirable to mitigate the costs of additional verification and the unnecessary deferral of donors.

In summary, the Alinity s and Alinity i systems and the assays intended for them are in full alignment with the requirements and standards for application of blood screening in Poland. Testing positive local samples provides information on the clinical sensitivity compared to the systems used previously. The agreement between the specificity results from the evaluation and those observed during the first 12–18 months of routine use of Alinity s and Alinity i systems shows that the Polish validation procedure is efficient and practical.

CRediT authorship contribution statement

Aneta Kopacz: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **Dorota Kubicka-Russel:** Data curation, Formal analysis, Methodology, Resources, Visualization. **Grzegorz Liszewski:** Data curation, Methodology, Validation. **Alicja Bukowska:** Methodology, Resources. **Sylwia Samek:** Methodology, Resources. **Dorota Malka:** Methodology, Resources. **Magdalena Łętowska:** Supervision. **Piotr Grabarczyk:** Conceptualization, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data availability

Data will be made available on request.

Acknowledgement

Open access publishing costs has been paid by Abbott.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00364.

References

[1] World Health Organization. Screening Donated Blood for Transfusion-Transmissible Infections: Recommendations, World Health Organization, 2009. https://apps.who.int/iris/handle/10665/44202.
[2] OBWIESZCZENIE MINISTRA ZDROWIA z dnia 30 marca 2021 r. w sprawie wymagań dobrej praktyki pobierania krwi i jej składników, badania, preparatyki, przechowywania, wydawania i transportu dla jednostek organizacyjnych publicznej służby krwi http://dziennikmz.mz.gov.pl/api/DUM_MZ/2019/25/journal/5348.
[3] Vockel, et al., Specificity of automated blood screening assays developed for the new Alinity s system, Vox Sang. 112 (S1) (2017) 162 (P-289).
[4] Sickinger, et al., Performance characteristics of the four screening assays, HBsAg Qualitative II, Anti-HCV, HIV Ag/Ab Combo and Syphilis on Abbott's new Alinity i system, Vox Sang. 112 (S1) (2017) 162 (P-289):163(P-293).
[5] I. Guetta, V. Petit, I. Ferrando, L. Maisonneuve, J. Mittler, D. Lusina, Analytical characteristics of Vitros 3600 analyzer, Ann. Biol. Clin. 68 (4) (2010 Jul-Aug) 465–472.
[6] G. Hersmann, C. Helbig, K. Linke, H. Thomas, C. Guenther, H. Kroll, Validation of the Alinity s assays for detection of HBsAg, HIV Ag/Ab, anti-HCV and anti-HBc, Vox Sang. 114 (Suppl. 1) (2019), 5–240, P-224.
[7] V. Zevgolis, C. Mantatzidou, G. Kastaniotis, D. Christopoulou, A. Milaiou, E. Koutroulaki, K. Stamoulis, Evaluation of the ABBOTT ALINITY S system and estimation of the specificity of the novel HBsAg, HIV Ag/Ab COMBO, anti-HCV and HTLV-I/II assays versus PRISM using routine blood donor samples from Greece, Vox Sang. 114 (Suppl. 1) (2019) 241.

[8] A. Van Weert, M. Koot, E. Bakker, Experience with the ALINITY s assays after 5 months of routine use in high-volume blood donation screening, Vox Sang. 114 (Suppl. 1) (2019), 5–240: 4D-S26-04.
[9] C. Tinguely, M. Hotz, C. Niederhauser, Evaluation of the usability of the newly launched Alinity s and the specificity of HIV COMBO, anti-HCV, HBsAg and Syphilis in a blood donor screening setting, Vox Sang. 114 (Suppl. 1) (2019), 5–240: 14D-S26-03.
[10] R. Gonzalez Diez, A. Richart Lopez, M. Ruiz Tovar, A. Arruga Manzano, J. Rodriguez-Gambarte, Y. Hermenegildo Lopez, I. Lucea Gallego, R. Alenda Asensi, L. Barea Garcia, Evaluation of the specificity provided by Alinity s versus Prism (HBsAg, HCV-Ab, HIV-Ag/Ab) and Architect syphilis-Ab, Vox Sang. 115 (Suppl. s1) (2020), 3–387: P-331.
[11] M. Stojic Vidovic, A. Hecimovi, M. Berendika, M. Miletic, D. Grubesi, I. Babic, M. Strauss Patko, I. Jukic, Experience with Alinity s system after one year of routine work, Vox Sang. 115 (Suppl. s1) (2020), 3–387: P-337.