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An independent reader system that parallels critical steps of full automation capability for immunohematology testing

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ABSTRACT

Introduction: Use of fully automated solutions to perform analysis of immunohematology tests is highly desirable as it delivers an improved level of safety and security of results. However, full automation may not be feasible financially and practically in all circumstances. A solution that addresses most of the process step hazards of manual testing can assist in achieving a higher level of confidence in and safety of test results.
Methods: The study utilized a column agglutination technology (ORTHO BioVue ® System) to test a variety of samples on the ORTHO VISION ® Analyzer and compare to the reader capability of the ORTHO OPTIX™ Reader. Both direct agglutination and direct/indirect antiglobulin test methods were evaluated. The data was analysed for per cent agreement and for concordance at the lower bound 95% confidence interval. The acceptance criteria for concordance for direct agglutination was $\geq$ 99.4% and for indirect agglutination was $\geq$ 98.0% at a lower bound 95% confidence interval.
Results: There were 13,805 columns producing 5174 interpreted tests for direct agglutination and 5998 columns producing 2958 interpreted direct/indirect antiglobulin tests evaluated. Testing demonstrated that direct agglutination and direct/indirect antiglobulin testing generated greater than 99% concordance between the fully automated instrument system and the reader.
Conclusions: The reader exceeded the approval criteria set for the study which demonstrates the capability to address the desire for a solution that moves manual testing to an enhanced level which achieves improved safety and security in immunohematology testing.

1. Introduction

The elimination of manual interaction in the processing of immunohematology (IH) tests has been recognized as a significant measure to decrease the risk of error potential and establish improved safety and security of test results. Over time incremental advances have been made to minimize the risk associated with using essential high skilled resources required for manual tube techniques. The progression along the path to fully automate testing was spurred with the invention of column-based technology that addressed several aspects of tube-based tests that drives error potential including multiple tubes to manage, multiple reagent additions, individual technique influence and finally unstable reactions. The column-based tests offered significant improvement in consistency and standardization. However, not all risks were minimized but the realization of a path to automating IH now had traction to address other key process control points. Further trailblazing of the automation pathway came with innovations of precision pipetting systems, reader development and software, both to operate the instrument and user interface. Ultimately, automating testing progressed with the integration of these technological advanced innovations into instrumentation combined with the column-based technology. Using a fully automated instrument provides the highest level of mitigation of potential for error. The evidence that automating as much as possible has been supported using failure mode effect analysis in transfusion medicine [1,2] to evaluate process, identify defect opportunities and ultimately reduce risks for the potential for error [3]. However, fully automated instruments are not always economically or practically feasible in all circumstances, therefore, a solution that addresses as

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many of the critical control points in the process to meet the needs of facilities endeavoring to enhance transfusion safety is essential. Innovative features along the automation continuum have provided the foundation that could address this issue. A reasonable alternative to full automation that would satisfy this expectation would address linkage of patient sample to reagents tested through as many process steps to reaction grading/ interpretation, all captured through system software. This would complement those healthcare systems that operate in a hub and spoke model with semi-automated systems that are analogous to the automation at the hub level. Table 1 identifies key critical control points along the process of testing using manual, semiautomated and fully automated solutions.

Based on user informed criteria for the development of ORTHO VISION® (VISION) and ORTHO VISION® Max Analyzers (Ortho Clinical Diagnostics, Raritan, NJ, US), fully automated systems for immunohematology testing, an opportunity to apply key attributes common to these instruments to a semi-automated solution was explored [4]. Using this concept ultimately provided a solution for those who wanted as many of the features of full automation in a semi-automated system. One of the key attributes leveraged was the imaging processing software in software architecture, interface, and design. The data parameter file used on the new reader required only minor updates to address the few minor differences between the fully automated system and the semi-automated reader. The geometry of the reader is the same, however the cassette holder is within the reader and will rotate the cassette for imaging both sides. The illumination aspect of imaging is consistent across both systems which is critical to standardization of the image capture. As part of the column grading process, which is equivalent to the grading process used on the fully automated ORTHO VISION platform, a check of red cell quantity in the column is inherent within the software. Using these key system imaging components and software functionality, the ORTHO OPTIX™ Reader System (Ortho Clinical Diagnostics, Raritan, NJ, US) was developed.

An internal and external site testing protocol was designed to capture both internal site data and data from a clinical laboratory environment experience. The data generated was used to establish that the OPTIX Reader delivers a performance level at least equivalent to the ORTHO VISION through evaluation of concordance between the new reader and the fully automated instrument.

Table 1
Critical Control Points - Level of Automated Control Across Manual, Semi-automated and Fully Automated Solutions.

Critical Control Point	Manual Testing	Semi-Automated Reader	Fully Automated
Sample Identity	Manual Visual	Barcode to SW	Automated Barcode to SW
Reagent Identity	Manual Visual	Barcode to SW	Automated Barcode to SW
Sample Addition	Manual Visual	Manual Visual Precision Pipettor	Automated Precision Pipettor
Reagent Addition	Manual Visual	Manual Visual	Automated Precision Pipettor
Test Processed	Manual Visual	Manual Visual	Automated Process Control
Test Centrifuged	Manual Visual	Automated	Automated
Test Reaction Read	Manual Visual	Reader	Automated Reader
Test Reaction Recorded	Manual Visual	Software	Software
Test Interpreted	Manual Visual	Software	Software
Computer Entry	Manual Visual	LIS Interface	LIS Interface
Final Record	LIS	SW- Image and test result stored	SW stores process steps, images and test results

2. Materials and methods

Table 2 lists the ORTHO BioVue® System ((BioVue) Ortho Clinical Diagnostics, Raritan, NJ, US) cassette types, test method validated, number of columns tested and number of interpreted tests at the internal testing site. Both direct agglutination tests and direct/indirect antiglobulin tests were executed at the internal testing site. Direct agglutination testing involved tests where there was no requirement for an antiglobulin reagent to be employed. All tests having a need for an antiglobulin reagent were part of the group of indirect antiglobulin tests. ORTHO 3–4% and 0.8% reagent red blood cell products were used in performing tests including reagent red blood cell products depending on site study protocol. Test samples included: fresh (within 7 days of collection) centrifuged whole blood, frozen plasma, packed red blood cells and cord blood samples.

Testing at the external site, located in a large blood center in Germany, completed both direct agglutination tests and indirect antiglobulin tests on donor samples available from their general sample populations. This study employed the use of the 3–4% ORTHO reagent red blood cells. Table 2 lists the BioVue cassette types, test method validated, number of columns tested and number of interpreted tests at the external testing site.

On each day of use, quality control of the cassettes, reagents and instruments was performed on each reagent and cassette combination tested. Results of quality control testing of quality control samples were required to demonstrate stated acceptance criteria before study testing was executed.

2.1. The internal study

Testing included direct agglutination - ABO/RH, antigen phenotyping and indirect antiglobulin - antibody detection, antibody identification, crossmatch and direct antiglobulin test (DAT). Two VISION Analyzers and three OPTIX Reader Systems were used. The reader used with each automated instrument was rotated each test day so that all readers were used with both VISIONs. Samples were processed and read on the automated instrument and immediately read on the paired reader. Results were evaluated for concordance on column-by-column basis and the interpreted result basis.

2.2. The external study

Testing included direct agglutination - ABO/RH, antigen phenotyping and indirect antiglobulin - antibody detection, and crossmatch tests. One VISION Analyzer and two OPTIX Readers were used. Samples were processed and read on the automated instrument and immediately read on the reader. Results were evaluated for concordance on column by column and the interpreted result basis.

1.2.3 For this study, the ORTHO VISION instrument was considered the predicate device to which the OPTIX reader was compared. For both sites, the acceptance criteria for concordance for direct agglutination was $\geq$ 99.4% at a lower bound 95% confidence interval and for direct and indirect antiglobulin was $\geq$ 98.0% at a lower bound 95% confidence interval.

Any discordant column was repeated on the same original systems as well as manually on the ORTHO BioVue System – ORTHO Workstation with the following approach. Each original cassette/ column with a discordant result was manually read. Then the discordant sample was tested with the same lot of reagents and cassettes using the manual BioVue test method followed by a manual read by a trained operator and immediately read on the OPTIX reader. Additionally, the discordant sample was retested on the same VISION instrument and the cassette then re-read on the same OPTIX reader. The manual retest served as the referee of the final result.

Table 2
Cassette Type Tested, Test Method, Columns Tested, Interpreted Tests - Internal and External Test Sites.

ORTHO BioVue Cassette Type	Cassette Configuration	Test Method	Internal Tests		External Tests	
			Columns Tested	Interpreted Tests	Columns Tested	Interpreted Tests
ABO-Rh/Reverse	A/B/D/CT/RD/RD	DA	2994	ABO: 499 RhD: 499	3108	ABO: 518 RhD: 518
Rh/K	C/E/c/e/K/CT	DA	900	Rh Pheno: 150 K: 150	180	Rh Pheno: 30 Kell:30
Rh/K II (second clone)	C/E/c/e/K/CT	DA	924	Rh Pheno: 154 K: 154	168	Rh Pheno: 28 Kell:28
ABDD/K; ABD/CDE: Newborn	A/B/D/D/K/CT; A/B/A,B/D/CDE/CT A/B/A,B/D/CT/IgG	DA	2940	490		
ABO-Rh	A/B/D x 2	DA	636	ABO: 212 RhD: 212		
AHG Polyspecific/ Neutral	AHG-PS X 3 / Neut x 3	IAT/ENZ	Neut:300	AbScr: 100		
Reverse Diluent (RD)	RD x 6	DA	2059	ABO: 605 ISXM: 207 *D: 37		
DAT/IDAT	IgG/C3d/CT x 2	DAT/ IAT	210	DAT: 50 AC: 20		
AHG Polyspecific	AHG-PS x 6	IAT	300	AbScr: 100	1554	Ab Scr: 518
AHG Anti-IgG	IgG x 6	IAT	1403	AbScr: 100 AbID: 16 Major XM: 234 Minor XM: 225 DAT: 117	210	Major XM: 210

SW - Software, LIS - Laboratory Information System DA - Direct Agglutination, RD - Reverse Diluent, AHG - Anti-Human Globulin (antiglobulin), DAT - Direct Antiglobulin Test, IAT - Indirect Antiglobulin Test, ENZ - Enzyme, XM - Crossmatch, ISXM - Immediate Spin Crossmatch, AC – Autocontrol, *RhD Typing with ORTHO Sera Anti-D(DVI)

3. Results

The internal test site comparison testing by direct agglutination (Table 3) was executed on a total of 2317 samples generating 10349 column results (5262 positive / 5087 negative) that were analyzed for concordance. The concordance achieved was 100%. The overall concordance at the lower bound 95% confidence interval (CI) was 99.9% for direct agglutination. The overall agreement of 4022 interpreted tests at the interpreted result level was 100.0% with a 99.9% concordance at the lower bound 95% confidence interval.

Comparison of direct and indirect antiglobulin testing was executed on a total of 2280 samples (Table 4) generating 4234 columns (621 positive / 3606 negative / 7 discordant) with an overall concordance of 99.8% with a 99.7% concordance at the lower bound 95% confidence interval.

Of the discordant test results (Table 5), there was one test positive by VISION full automation and negative by the OPTIX reader, while six tests that had one negative column on the VISION were positive on the OPTIX System. This represents 0.16% of the total columns evaluated in the indirect antiglobulin tests. All discordant results demonstrated very weak positive reactivity. Both direct and indirect antiglobulin testing achieved acceptance criteria. The overall agreement of 2230 interpreted tests at the interpreted result level was 99.7% with a 99.4% concordance at the lower bound 95% confidence interval thus meeting the acceptance criteria of 98.0%.

All discordant tests (Table 5) were graded by the imaging system at a 0.5 reaction initially on either the VISION Analyzer or the OPTIX system. All tests were negative when tested by the referee BioVue manual test except one that had demonstrated an initial positive result using the OPTIX system and also tested positive on the manual BioVue Workstation test. The discordant results remained in the analysis of concordance. There was no assignable root cause for the discordant results identified. Indirect antiglobulin testing achieved acceptance criteria.

The external study site comparison testing by direct agglutination (Table 6) was executed on a total of 577 samples generating 3456 column results (1600 positive / 1856 negative) that were analyzed for concordance. The concordance achieved was 100%. The overall concordance at the lower bound 95% confidence interval (CI) was 99.9% for direct agglutination. Overall interpreted test achieved a 100% agreement with a concordance at the lower bound 95% confidence interval at 99.7%. The overall agreement of direct agglutination tests of the 1152 interpreted tests at the interpreted result level was 100.0% with a 99.7% concordance at the lower bound 95% confidence interval. Comparison of indirect antiglobulin tests by indirect antiglobulin testing (Table 6) was executed on a total of 799 samples generating 1764 columns (122 positive/1642 negative) with an overall concordance of 100.0% with a 99.8% concordance at the lower bound 95% confidence interval. There were no discordant tests. The indirect antiglobulin testing achieved acceptance criteria.

The overall agreement of 728 indirect antiglobulin tests at the interpreted result level was 100.0% with a 99.6% concordance at the lower bound 95% confidence interval. Table 7.

4. Discussion

Fully automated systems, like the ORTHO VISION Analyzer, offer the

Table 3
Overall Column Result % Agreement for Direct Agglutination Tests – Internal.

ORTHO OPTIX						
ORTHO VISION	2317 Samples	Positive	Negative	Total	Concordance	
	Positive	5262	0	5262	Overall Concordance %	100%
	Negative	0	5087	5087	Lower Bound of the 95% Confidence Interval	99.9%
	Total	5262	5087	10349	Direct Agglutination Acceptance Criteria	≥99.4%

Table 4
Overall Column Result % Agreement for Direct and Indirect Antiglobulin Tests-Internal.

ORTHO OPTIX						
ORTHO VISION	2280 Samples	Positive	Negative	Total	Concordance	
	Positive	621	1	622	Overall Concordance %	99.8%
	Negative	6	3606	3612	Lower Bound of the 95% Confidence Interval	99.7%
	Total	627	3607	4234	Direct/Indirect Antiglobulin Acceptance Criteria	≥ 98.0%

Table 5
Discordant Results by Column (Reagent) for Indirect Antiglobulin Tests Confirmed by Manual BioVue Testing (Internal Testing).

Test	Test Profile	Reagent	VISION Initial Result	OPTIX Initial Result	Retest Manual BioVue Read/ Reread OPTIX	Retest VISION Read/ Reread OPTIX
Antibody Screen	08 AbScr Surg IgG	Anti-IgG	0	0.5	0/0	0/0
Antibody Screen	4 AbScr Surg Poly	Anti-IgG, -C3b/d	0	0.5	0/0	0/0
Autocontrol	AC 4 Auto Poly	Anti-IgG, -C3b/d	0	0.5	0/0	0/0
Antibody Screen Ficin	4 BVSF Poly/ Neut	Neutral	0	0.5	0/0	0/0
Crossmatch	08 Maj XM Poly	Anti-IgG, -C3b/d	0.5	0	0/0	0/0
Crossmatch	08 Maj XM Poly	Anti-IgG, -C3b/d	0	0.5	0.5/0.5	0/0
Crossmatch	08 Maj XM Poly	Anti-IgG, -C3b/d	0	0.5	0/0	0/0

transfusion medicine laboratory key advantages of end-to-end process control (from sample load to result output). The fully automated system can virtually monitor almost every aspect of the process and capture with precision that information on a sample-by-sample basis. This provides for the rationale that full automation delivers a higher level of safety and security, particularly if interfaced to data management systems and laboratory information systems eliminating manual transcription errors. Biological safety is another aspect that a full automation system provides as human interaction is limited to sample load and removal. With full automation, turnaround time can be an advantage over manual or semiautomated approaches since continuous load and processing generally provides for a more consistent predictable test turnaround time.

In a study by Dara et. al. [5], that evaluated 2631 immunohematology test profiles (ABO/Rh, antibody detection, antibody identification, crossmatch and DAT) for agreement, compared the ORTHO VISION with their laboratory's predicate ORTHO BioVue System. Testing in the BioVue system was completed with all steps being executed manually by an operator including visual reading of test results. Testing demonstrated 100% agreement in all, except for two profiles tested; 1) one weak A blood type detected on VISION but not detected in the manual BioVue test, 2) one weak anti-M identified in the antibody identification test on the VISION but not on the BioVue test. This study also evaluated turn-around-time of testing which demonstrated the turn-around-time to achieve completed results, depending on testing profile, on average took 1.5–3 times longer by the manual BioVue method compared to the automated approach.

However, there is a substantial cost differential between a fully automated and semiautomated systems, usually as much as five times that of a semi-automated solution. For that reason, the economic feasibility of deploying full automation systems into every laboratory is unachievable. However, solutions that combine key features of full automation and information technology can help address this need. Additionally, as hub and spoke models of health care delivery networks evolve, consistency, safety and security between facilities can be achieved using full automation in the larger transfusion service with satellite smaller facilities employing the paired semi-automated solution.

The OPTIX Reader System was developed using common components and software features from an established fully automated system (ORTHO VISION Analyzer). The intent was to minimize the number of human interactions so that key automation features would provide for immunohematology / transfusion service laboratories the desired increased security of their test results [6]. The ORTHO OPTIX System delivers benefits in four key and critical areas, 1) positive sample identification with reagent cassette linkage, 2) consistent reader reaction grading with image capture, 3) result interpretation with 4) data storage. The OPTIX system or multiple systems can be interfaced to the LIS or through a single data management system such as the ORTHO CONNECT™ (Ortho Clinical Diagnostics, Raritan, NJ, US). This

Table 6
Overall Column Result % Agreement for Direct Agglutination Tests- External.

ORTHO OPTIX						
ORTHO VISION	577 Samples	Positive	Negative	Total	Concordance	
	Positive	1600	0	1600	Overall Concordance %	100%
	Negative	0	1856	1856	Lower Bound of the 95% Confidence Interval	99.9%
	Total	1600	1856	3456	Direct Agglutination Acceptance Criteria	≥99.4%

Table 7
Overall Column Result % Agreement for Indirect Antiglobulin Tests- External.

ORTHO OPTIX						
ORTHO VISION	799 Samples	Positive	Negative	Total	Concordance	
	Positive	122	0	122	Overall Concordance %	100.0%
	Negative	0	1642	1642	Lower Bound of the 95% Confidence Interval	99.8%
	Total	122	1642	1764	Indirect Antiglobulin Acceptance Criteria	≥ 98.0%

translates into improved safety and security, delivering a reliable immunohematology test results that minimizes the influence of manual interaction hazards through a semi-automated solution.

Using a comparison to a predicate device, the ORTHO VISION, the ORTHO OPTIX Reader was evaluated by two different laboratories comparing test results across the same individual laboratory sample population for concordance. The testing in this study, completed at both sites, demonstrated that the concordance acceptance criteria was achieved by the OPTIX reader. The clinical laboratory experience input to the performance of the OPTIX reader was a valuable contribution to the study assuring that the reader system's capabilities met actual user expectations.

CRediT authorship Contribution Statement

Tony S. Casina: Writing – original draft, Writing – review and editing. **Sawyer, Bridget:** Methodology, Formal analysis. **Wycallis, Joe:** Conceptualization, supervision. **Ernst, Lauren:** Methodology, Formal analysis. **Wilson-Colley, Amy:** Validation, Formal analysis, Investigation.

References

[1] Mora A, Ayala L, Bielza R, Gonzalez FA, Villegas A. Improving safety in blood transfusion using failure mode and effect analysis. Transfusion 2019;59:516–23. https://doi.org/10.1111/trf.15137.

[2] Lu Y, Teng F, Zhou J, Wen A, Bi Y. Failure mode and effect analysis in blood transfusion: a proactive tool to reduce risks. Transfusion 2013;53:3080–7. https://doi.org/10.1111/trf.12174.

[3] Han TH, Kim MJ, Kim S, Kim HO, Lee MA, Choi JS, et al. The role of failure modes and effects analysis in showing the benefits of automation in the blood bank. Transfusion 2013;53:1077–82. https://doi.org/10.1111/j.1537-2995.2012.03883.x.

[4] Chung HJ, Hur M, Choi SG, Lee HK, Lee S, Kim H, et al. Benefits of VISION Max automated crossmatching in comparison with manual crossmatching: a multidimensional analysis. PLOS ONE 2019:1–13. https://doi.org/10.1371/journal.pone.0226477.

[5] Dara RC, Tiwari AK, Mitra S, Acharya DP, Aggarwal G, Arora D, et al. Comparison of a column agglutination technology-based automated immunohematology analyzer and a semiautomated system in pretransfusion testing. Asian J Trans Sci 07/ 2019;13(2):115. https://doi.org/10.4103/ajts.AJTS_116_17'.

[6] South SF, Casina TS, Li L. Exponential error reduction in pre transfusion testing with automation. Transfusion 2012;52:81S–7S. https://doi.org/10.1111/j.1537-2995.2012.03816.x.