A Multi-Site Evaluation for Performance of Fully Automated Antigen Typing

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BACKGROUND/CASE STUDIES

Evaluation of an immunohematology testing system is necessary to show that the performance of the instrument demonstrates equivalence from a method-based perspective when compared to results of a predicate method or instrument. The ORTHO VISION® Analyzer is designed to fully automate extended antigen typing using the ID-MTS™ Gel Card (GEL) test along with a variety of ORTHO™ Sera Blood Grouping Reagents specificities. A multi-site study was conducted to evaluate the performance of the ORTHO VISION automated red cell antigen typing utilizing ORTHO Sera reagents with specific ID-MTS Gel Cards compared to the predicate test, the manual ID-MTS GEL test using the ORTHO™ Workstation. Testing included reproducibility, a measure of total precision and repeatability, which evaluates within-run test precision. This provides insight into consistency of result and degree of variability of reactivity of the automated method of testing.

STUDY DESIGN/METHODS

Testing occurred across three laboratory study sites. Method comparison testing of the thirteen antisera was conducted using the automated and manual tests on samples acquired from the sites' routine workload. Quality control testing utilized 0.8% Reagent Red Blood Cells (0.8% SELECTOGEN®, 0.8% SURGISCREEN® and 0.8% RESOLVE® Panels) for positive and negative controls. Depending on the ORTHO Sera being tested, an ID-MTS Anti-IgG Gel Card or the ID-MTS Buffered Gel Card was used (Table 1).

Table 1: Antisera Specificity, Source, Clone Identity, Gel Card Type, Test Technique and Sample Number Tested

ORTHO Sera Specificity	Antisera Source	Clone(s)	ID-MTS Gel Card	Test Technique	Samples Tested
Anti-Jk^a	Monoclonal	P3HT7	Buffered Gel	Papain, 15' RT	1250
Anti-Jk^b	Monoclonal	P3.143	Buffered Gel	Papain, 15' RT	1241
Anti-Fy^a	Monoclonal	DG-FYA-02	Anti-IgG	15' 37°C, AHG	1216
Anti-Fy^b	Human	Polyclonal	Anti-IgG	15' 37°C, AHG	1270
Anti-S	Monoclonal	P3S13JS123	Anti-IgG	15' 37°C, AHG	1258
Anti-s	Monoclonal	P3YAN3	Anti-IgG	15' 37°C, AHG	961
Anti-K	Monoclonal	MS-56	Buffered	Immediate Spin, RT	938
Anti-Le^a	Monoclonal	LEA1	Buffered Gel	Papain, 15' RT	1275
Anti-Le^b	Monoclonal	LEB1	Buffered Gel	Papain, 15' RT	1232
Anti-P1	Monoclonal	650	Anti-IgG	Immediate Spin, RT, AHG	1299
Anti-N	Monoclonal	BO3	Buffered Gel	Immediate Spin, RT	1229
Anti-D (IAT)	Monoclonal	LDM3/ESD1	Anti-IgG	15' 37°C, AHG	1283
Anti-D (DVI)	Monoclonal	ESD1M	Buffered Gel	Immediate Spin, RT	1255

AHG - Anti-human globuin (antiglobulin)
RT – Room temperature

Each antisera specificity was tested for reproducibility/repeatability on five non-consecutive days with two runs of two test replicates of the same antigen positive and negative red cells. Method comparison concordance test results were assessed by comparison of interpreted tests to determine % concordance between the automated test and the manual test at the one sided lower 95% confidence bound (LCB95) for the positive % agreement (PPA) and negative % agreement (NPA). Resolution of discordant tests, defined as positive by one test and negative in the comparative method, included repeat testing by both methods and a test by an independent resolution tube test method. Any test that was discordant between comparison methods using the antiglobulin (Anti-IgG) Gel card was tested by the direct antiglobulin test. Direct antiglobulin test (DAT) positive samples were removed from concordance analysis for those antisera requiring an antiglobulin test method. If a discordant test result was investigated by the site and found not reproducible by the manual method, it was deemed a manual use error and excluded from concordance analysis. The LCB95 acceptance criteria for a combined PPA and NPA was ≥ 99.0%.

RESULTS/FINDINGS

Concordance testing demonstrated an overall % agreement (OA) of ≥99.0% at LCB95 across 12 of the antisera. Anti-P1 was at 98.8%. See Table 2 for positive percent agreement (PPA) and negative percent agreement (NPA).

Table 2: Concordance Results of ORTHO Sera/ORTHO VISION Analyzer

Antisera	Jk^a	Jk^b	Fy^a	Fy^b	S	s	K	Le^a	Le^b	P1	N	D(IAT)	D(VI)
#Pos. Tests	635	637	616	641	620	653	314	611	632	675	606	655	633
#Neg. Tests	615	604	593	623	634	308	624	664	600	619	623	626	622
PPA 95% LCB-%	99.3	99.3	99.5	99.5	99.5	99.5	99.1	99.2	99.5	99.6	99.5	99.5	99.5
NPA 95% LCB-%	99.2	99.5	99.5	99.2	99.5	99.0	99.5	99.1	98.5	97.9	99.2	99.5	99.5
OA 95% LCB-%	**99.6**	**99.6**	**99.6**	**99.6**	**99.8**	**99.7**	**99.7**	**99.4**	**99.3**	**98.8**	**99.6**	**99.8**	**99.8**

There were 24 test results that were eliminated from final concordance analysis as the result of the presence of a positive DAT. Most of these samples showed weak positive (1+) DAT results. See Table 3.

Table 3: DAT Positive Samples Eliminated from Final Concordance Analysis

ORTHO Sera Specificity	# DAT + Samples
Anti-Fy^a	7
Anti-Fy^b	6
Anti-S	4
Anti-P1	5
Anti-D (IAT)	2

Any site identified manual error results were eliminated from concordance analysis. There were no discordant results with the following reagents Anti-Fy^a, -S, -s, -K, -D(IAT), and -D(VI). There were four samples that were later identified to be possible manual errors that remained in the final concordance analysis.

Table 4 shows the concordance analysis including DAT positive samples* which are now incorporated in the final approved commercial version of instructions for use of these 5 antisera.

Table 4: Concordance of Antisera including DAT Positive Samples

ORTHO Sera Specificity	Antigen Positive/ Negative	Number of Samples Tested	Comparator Analysis	
			% Agreement	One sided 95% CI
Anti-Fy^a	Positive	616	100.0	99.5
	Negative	600*	98.8	97.8
Anti-Fy^b	Positive	641	100.0	99.5
	Negative	629*	98.9	97.9
Anti-S	Positive	620	100.0	99.5
	Negative	638*	99.4	98.6
Anti-P1	Positive	675	100.0	99.6
	Negative	624*	98.1	96.9
Anti-D (IAT)	Positive	655	100.0	99.5
	Negative	628*	99.7	99.0

Discordant results are detailed in Table 5. One sample demonstrated positive reactivity with Anti-Fy^b on the automated system and was repeatable, however was nonreactive with manual test methods and found by genotyping to predict the sample as Fy(b+). Discordant results for the Anti-P1 were mostly attributed to very weak expression of P1. There were four samples that were tested with various anti-P1 reagents (various manufacturer/reagent source) with variable results seen; some reagent indicating the samples to be P1+ and other reagent as P1-. The presence of discrepancies with Anti-P1 reagents is not unexpected as the expression of the P1 antigen can vary substantially. For this reason, the 98.8% can be considered acceptable for reagent performance for the Anti-P1 being evaluated.

The remaining discordant results initially negative by manual testing and positive by automated testing were generally weak in reactivity and graded by the imaging system at a reaction grade of 1+. The images of these results were reviewed with most of the images identified as atypical of a positive reaction. A root cause for these results could not be identified.

Reproducibility and repeatability testing demonstrated 100% agreement and were within the ≤1+ result for variability in positive reaction grading. Reaction grades for positive tests for all antisera was at a minimum of 3+ except for Anti-Fy^b which had a 2-3+ reactivity range but not less than 2+.

Table 5: Discordant Sample Details

ORTHO Sera Specificity	Sample	Initial Test		Discordant Resolution Repeat Test			
		Manual Workstation	ORTHO VISION Analyzer	Manual Workstation	ORTHO VISION Analyzer	Resolution Tube Test	Comment
Anti-Jk^a	1	0	1+	0	0	0	Cell button disrupted inconsistent with + rxn - NRC
	2	1+	0	0	0	0	Possible manual error
Anti-Jk^b	1	2+	0	0	0	0	Possible manual error
Anti-Fy^b	1	0	1+	0	0	0	Predicted as Fy(b+) by genotype
Anti-Le^a	1	2+	0	0	0	0	Possible manual error
	2	0	1+	0	0	0	Cell button disrupted inconsistent with + rxn - NRC
	3	0	1+	0	0	0	Cell button slanted inconsistent with + rxn - NRC
Anti-Le^b	1	0	1+	0	1+	0	Cell button slanted inconsistent with + rxn - NRC
	2	0	1+	0	1+	0	Cell button slanted inconsistent with + rxn - NRC
	3	0	1+	NP	0	NP	Cell button slanted inconsistent with + rxn - NRC
	4	0	1+	0	Ind	0	Cell button slanted inconsistent with + rxn - NRC
Anti-P1	1	0	1+	0	?	0	Cell button slanted inconsistent with + rxn - NRC
	2	0	1+	0	1+	Positive	Variable positive/negative with various anti-P1 reagents- P1+^{w}
	3	0	1+	0	1+	Positive	Variable positive/negative with various anti-P1 reagents-P1+^{w}
	4	0	1+	0	0	Positive	Variable positive/negative with various anti-P1 reagents-P1+^{w}
	5	0	1+	0	1+	1+	Weak P1 expression – P1+^{w}
	6	0	1+	0	1+	0	Cell button disrupted inconsistent with + rxn - NRC
	7	0	2+	0	0	0	Weak agglutination to one side of the column - NRC
Anti-N	1	0	2+	1+	2+*	3+	Possible manual error

NRC - A root cause could not be assigned to the reactivity seen.
NP - Not performed
rxn - Reaction

CONCLUSIONS

The multi-site evaluation demonstrated a high level of concordance of ORTHO Sera reagents in the comparison of testing between the ORTHO VISION Analyzer and the manual system ID-MTS Test. The benefits of automated testing can be achieved using extended antigen typing on a fully automated test platform providing for improved efficiency, reduced potential for error and complete traceability of all test processing. Additional enhanced security is gained through electronically captured test results and reaction grade images. The value this brings to the blood bank/transfusion service in safety and productivity is substantial considering current challenges in workforce resources.

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QUOTIENT

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