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# Extended Antigen Typing on a Fully Automated Immunohematology Analyzer

Tony S. Casina[1], Brad Roughsedge[2], Janine S. Robb[3], Emma R. Waite[3], Scott Burrell[3] and Robert Witkowski[2]

[1]TSC Consulting, Newtown, PA, [2]Ortho Clinical Diagnostics, Rochester, NY, [3]Quotient, Edinburgh, United Kingdom

## BACKGROUND

Extended antigen typing, mainly executed using manual techniques in tube-based tests, has many failure modes. Testing a single sample with multiple antisera or a single antiserum with many samples along with variable reagent methodology are major hazard contributors amongst the many potentials for error. Automation of immunohematology testing offers considerable risk reduction by minimizing the many error opportunities through process control.

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® Max 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 Reagent specificities. Testing reproducibility, a measure of total precision and repeatability, 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

This study involved testing of 13 ORTHO Sera using ID-MTS Gel Cards (Anti-IgG/Buffered) on the ORTHO VISION Max Analyzer to show concordance to a predicate device, the ORTHO VISION® Analyzer. 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).

Three centers participated in the study with samples coming from their routine sample populations. Each sample was tested with the same lot of antisera on both instruments. Discordant tests (DT) were retested with both systems and a resolver test method, if discrepant, on retest. Reproducibility/repeatability studies were conducted to show consistency of reaction grade by testing a known positive and known negative sample on 5 non-consecutive days, 2 times with 2 replicates within the day. Quality Control was accomplished by testing selected antigen positive/negative controls from 0.8% ORTHO RESOLVE® Panels. Each antiserum was evaluated for concordance using a one sided lower 95% confidence bound (LCB95) calculation. The positive % agreement (PPA), negative % agreement (NPA) and overall % agreement (OPA) were calculated. Acceptance criteria was set at LCB95% at greater than or equal to 99% for OPA.

**Table 1: Antisera Specificity, Source, Clone Identity, Gel Card Type, Test Technique and Number of Samples 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 | 1246 |
| Anti-Jk$^{b}$ | Monoclonal | P3.143 | Buffered Gel | Papain, 15' RT | 1386 |
| Anti-Fy$^{a}$ | Monoclonal | DG-FYA-02 | Anti-IgG | 15' 37°C, AHG | 1248 |
| Anti-Fy$^{b}$ | Human | Polyclonal | Anti-IgG | 15' 37°C, AHG | 1220 |
| Anti-S | Monoclonal | P3S13JS123 | Anti-IgG | 15' 37°C, AHG | 1229 |
| Anti-s | Monoclonal | P3YAN3 | Anti-IgG | 15' 37°C, AHG | 975 |
| Anti-K | Monoclonal | MS-56 | Buffered | Immediate Spin, RT | 1003 |
| Anti-Le$^{a}$ | Monoclonal | LEA1 | Buffered Gel | Papain, 15' RT | 1261 |
| Anti-Le$^{b}$ | Monoclonal | LEB1 | Buffered Gel | Papain, 15' RT | 1316 |
| Anti-P1 | Monoclonal | 650 | Anti-IgG | Immediate Spin, RT, AHG | 1260 |
| Anti-N | Monoclonal | BO3 | Buffered Gel | Immediate Spin, RT | 1299 |
| Anti-D (IAT) | Monoclonal | LDM3/ESD1 | Anti-IgG | 15' 37°C, AHG | 1245 |
| Anti-D (DVI) | Monoclonal | ESD1M | Buffered Gel | Immediate Spin, RT | 1346 |

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

## RESULTS/FINDINGS

All ORTHO Sera met the overall % agreement concordance LCB95 criteria of 99% or greater. There were 15 tests with various antisera that were impacted by the presence of a positive direct antiglobulin test (DAT), all of which graded as 1+ on the image analysis. Those ORTHO Sera that use Anti-IgG Gel cards and had a DAT+ result impact on the agreement are indicated with an *. (Table 2)

**Table 2: Ortho Sera Concordance Data on ORTHO VISION Max**

| ORTHO Sera | Jk$^{a}$ | Jk$^{b}$ | Fy$^{a}$ | Fy$^{b}$ | S | s | K | D(IAT) | D(VI) | Le$^{a}$ | Le$^{b}$ | P1 | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #Positive Tests | 636 | 689 | 630 | 607 | 618 | 675 | 308 | 609 | 650 | 602 | 713 | 659 | 690 |
| #Negative Tests | 610 | 697 | 618 | 613 | 611 | 300 | 695 | 636 | 696 | 659 | 603 | 601 | 609 |
| PPA 95%CI% | 99.3 | 99.6 | 99.5 | 99.5 | 99.5 | 99.6 | 99.0 | 99.5 | 99.5 | 99.5 | 99.6 | 98.6* /99.0 | 99.6 |
| NPA 95%CI% | 99.5 | 99.3 | 99.0* /99.5 | 98.1* /99.2 | 99.0* /99.5 | 99.0 | 99.1 | 98.6* /99.5 | 99.6 | 99.1 | 98.3 | 99.2 | 99.0 |
| OPA 95%CI% | 99.6 | 99.7 | 99.8 | 99.1 | 99.8 | 99.6 | 99.4 | 99.8 | 99.8 | 99.5 | 99.2 | 99.4 | 99.5 |
| OPA* 95%CI% | NA | NA | 99.5* | 99.0* | 99.5* | NA | NA | 99.3* | NA | NA | NA | 99.2* | NA |
| DAT+ | - | - | 2 | 5 | 2 | 0 | - | 4 | - | - | - | 2 | - |

There were 32 discordant test results in 16,034 tests seen in 11 of 13 reagents including 15 DAT+ results. Five of the discordant tests were atypical reactions, 7 had no root cause identified and the remaining were due to weakened/variant expression of antigen, 1 Fy(b+$^{w}$), 2 P1+$^{w}$ and 2 N+ (1 weak). The Fy(b+$^{w}$) and 1 M+N+ sample were confirmed with molecular tests. (Table 3). The reproducibility/repeatability tests demonstrated 100% agreement, between sites, occasions and instruments, and were within the ≤1+ result for variability in positive reaction grading.

**Table 3: Discordant Sample Detail**

| ORTHO Sera Specificity | Sample | Initial Test | | Discordant Resolution Repeat Test | | | |
|---|---|---|---|---|---|---|---|
| | | ORTHO VISION Analyzer | ORTHO VISION Max Analyzer | ORTHO VISION Analyzer | ORTHO VISION Max Analyzer | Resolution Tube Test | Comment |
| Anti-Jk$^{a}$ | 1 | 0 | 1+ | 0 | 0 | 0 | Cell button disrupted inconsistent with + rxn - NRC |
| Anti-Jk$^{b}$ | 1 | 0 | 1+ | 0 | 0 | 0 | Weak agglutination - NRC |
| Anti-Fy$^{b}$ | 1 | 0 | 1+ | Indeterminate | 0 | 1+ | Weakened Fy (b+$^{w}$) Predicted as Fy(a+b+) by genotype |
| Anti-K | 1 | 0 | 1+ | 0 | 0 | 0 | Weak agglutination - NRC |
| | 2 | 0 | 1+ | 0 | 0 | 0 | Weak agglutination - NRC |
| Anti-Le$^{a}$ | 1 | 0 | 1+ | 0 | 0 | Not tested | Cell button disrupted inconsistent with + rxn - NRC |
| | 2 | 0 | 1+ | 0 | 0 | 0 | Weak agglutination - NRC |
| Anti-Le$^{b}$ | 1 | 0 | 1+ | 0 | 0 | 0 | Cell button disrupted inconsistent with + rxn - NRC |
| | 2 | 0 | 1+ | 0 | 0 | 0 | Cell button disrupted inconsistent with + rxn - NRC |
| | 3 | 0 | 1+ | 0 | 0 | 0 | Cell button disrupted inconsistent with + rxn - NRC |
| | 4 | 0 | 1+ | 0 | 0 | 0 | Weak agglutination - NRC |
| | 5 | 0 | 1+ | 0 | 0 | 0 | Weak agglutination - NRC |
| Anti-P1 | 1 | 1+ | Indeterminate | Indeterminate | 0 | Positive | DAT negative. Variable positive/negative with various anti-P1 reagents- P1+$^{w}$ |
| | 2 | 1+ | 0 | Indeterminate | 1+ | 0 | DAT negative. Possible weak P1 antigen expression |
| | 3 | 0 | 2+ | 0 | 0 | 0 | DAT negative. Positive reactivity initially with ORTHO VISION Max-NRC |
| Anti-N | 1 | 0 | 1+ | 1+ | 1+ | Positive | Weak expression of N antigen. Predicted as M+N+ by genotype |
| | 2 | 0 | 1+ | 0 | 0 | Weak Positive | Weak expression of N antigen. Genotype not tested |

NRC - A root cause could not be assigned to the reactivity seen.
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 Max analyzer and the ORTHO VISION analyzer system using the ID-MTS Gel Test. The benefits of automated testing can be achieved using extended antigen typing on a fully automated test platform providing 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. Full automation to perform extended antigen typing provides improved process control compared to that achieved in manual extended antigen typing. The value this brings to the blood bank/transfusion service in safety and productivity is substantial considering current challenges in workforce resources. In addition, testing demonstrated a high level of in-run and overall precision of the testing. The safety and security delivered by automation for routine IH testing can benefit extended antigen typing.




Ortho Clinical Diagnostics

QUOTIENT

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