ORIGINAL REPORT

Validity and reliability of serologic immunophenotyping of multiple blood group systems by ORTHO Sera with fully automated procedure

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The increase of immunization against blood group antigens has reinforced the need for automated extensive blood typing. The aim of this study was to assess both the validity and reliability of red blood cell (RBC) automated agglutination technology in testing for antigens of Kidd (Jk), Duffy (Fy), and MNS (Ss) blood systems. ORTHO Sera (Ortho Clinical Diagnostics, Raritan, NJ) anti-Jk^a, anti-Jk^b, Anti-Fy^a, anti-Fy^b, anti-S, and anti-s reagents were each tested on RBC samples previously typed. Replicates were performed on three separate testing sessions with three consecutive repetitions within each session, thus obtaining 486 test results. Accuracy was assessed by aggregate analysis of sensitivity, specificity, and area under the receiver operating characteristics curve (AUC). Reliability was estimated by a cross-classified mixed-effect logistic model. All reagents tested yielded optimal accuracy (100% for sensitivity and specificity, and 1.00 for AUC), except for anti-S, for which performance was slightly lower (98%, 100%, and 0.99, respectively), owing to misclassification of one sample in a single replicate. Anomalous automated measurements were recorded in 38 of 486 tests (7.8%), which then needed additional manual interpretation. Different sessions and samples were the major contributors to measurement failures (38% and 18%, separately). Order of repetitions and antigen specificity across replicates did not contribute to the risk of failures, although weak evidence of enhanced risk ($p < 0.10$) was observed with Jk testing. Automated RBC typing with ORTHO Sera reagents against antigens in the Kidd, Duffy, and MNS blood group systems displayed nearly 100 percent accuracy. However, a sizable number of replicates needed additional ad hoc interpretation, thus suggesting that the reliability could still be improved. Automated agglutination technology represents a viable option for phenotyping large volumes of samples. ***Immunohematology* 2018;34:140–147.**

Key Words: validation studies, blood group antigens, automation, agglutination tests, cross-classified mixed-effect models.

Allogeneic red blood cell (RBC) transfusions remain an essential part of supportive treatment in patients with hemoglobinopathies,[1] but they expose these patients to the risk of developing antibodies against foreign RBC antigens. The development of alloantibodies against RBCs may complicate transfusion therapy, causing hemolytic transfusion reactions, and present a challenge for finding compatible blood and for RBC crossmatching, thus ultimately jeopardizing transfusion safety. The frequency of alloimmunization is variable among patients—for example, it is apparently higher in patients with sickle cell disease than in individuals with myelodysplastic syndromes.[2] The risk of alloimmunization increases in parallel with the number of units transfused and is also higher in patients with previous alloimmunization.[2–4] The most frequent RBC alloantibodies are directed towards antigens in the Rh and Kell blood group systems.[5] The standards of the Italian Society of Immunohematology and Transfusion Medicine require all blood donors to be typed for extended antigens in the Rh and Kell systems,[6] which is necessary for transfusion support of patients with hemoglobinopathies.[1] Although this preventive measure has certainly contributed to lowering the risk of immunization against these antigens, the risk of immunization against other blood group antigens (e.g., Kidd [Jk], Duffy [Fy], and MNS [Ss]) persists.[7] When available donors who have been previously phenotyped for these antigens are lacking, managing immunized patients undergoing recurrent transfusions is challenging.[2]

Fewer than 1 in 500 white donors might be compatible with recipients having an antibody mixture of anti-c/E, anti-S, and anti-Jk^a.[8] Moreover, the increasing migration of people from Africa towards southern European countries will contribute to an increase in the number of patients with mixtures of specific antigen combinations. For instance, 60–70 percent of individuals migrating from sub-Saharan African regions do not express Fy antigens, which are instead present in higher percentages in individuals of European origin.[8]

A large array of automated systems is now available for serologic testing in transfusion services.[9–11] Fully automated

analyzers are essential components of many laboratory systems, and their gradual implementation into the laboratory routine has increased patient safety, enhanced standardization, and contributed to improving the workflow and reducing turnaround time.

ORTHO Sera (Ortho Clinical Diagnostics, Raritan, NJ) are sera for performing extended antigen phenotyping using the ORTHO BioVue System column agglutination technology (CAT) and are compatible for use on the fully automated ORTHO Analyzer (AutoVue Innova or Vision). ORTHO Sera have been recently introduced in our local laboratory for extended typing of blood donors. The adoption of a new technology or method in both the clinical and laboratory settings requires formal validation before widespread application to clinical practice.[12] *Validation* is the documented evidence that the process, equipment, facilities, and entire system operates within established parameters and can perform effectively and reproducibly, thus generating results that meet predefined quality specifications.[12] As part of validation, *qualification* is the action of verifying that personnel, equipment, or material work properly and deliver expected results.[12] In 2011, the British Committee for Standards in Haematology Blood Transfusion Task Force published guidelines for test validation and staff qualification.[13] More recently, the Italian National Blood Center has published new guidelines in support of process validation activities, entailing the collection of blood units and related compounds in Blood Transfusion Services.[14] Therefore, validation assesses both accuracy and reliability of a method. *Accuracy* represents a combination of sensitivity and specificity, which jointly provide the capability of a given method to generate results as close as possible to their true value. *Reliability* (i.e., precision) represents the ability of a method to provide similar results under equivalent (repeatability or intra-assay precision) or comparable (reproducibility or inter-assay precision) experimental conditions.

Therefore, the aim of this study was to validate the use of RBC automated CAT for testing Jk, Fy, and Ss blood group antigens using ORTHO Sera technology on the automated analyzer ORTHO AutoVue Innova.

Materials and Methods

Validation Plan and Study Design

According to our study design, successful validation was defined when the following endpoints could be satisfied:

- Accuracy: 100% sensitivity, specificity, and area under the receiver operating characteristics curve (AUC) identical to results previously obtained with the gold standard method (i.e., test tube).
- Reliability: confirmation of repeatability (intra-session) and reproducibility (inter-session).

As a secondary endpoint, we evaluated whether result reliability may be impaired by the following:

- Test replicate (session or repetition) or sample specificity.
- Blood group system (e.g., Kidd, Duffy, MNS).
- Antigen status (i.e., double-dose or single-dose phenotype).

We originally analyzed the technical aspects of validation by installation qualification (IQ) and operational qualification (OQ) of the analyzer. A performance qualification (PQ) was planned, by testing each variable in triplicate, as recommended by the Italian National Blood Center.[14] The variables in our qualification study were the antigen statuses of Jk, Fy, and Ss. Therefore, three types of samples were evaluated for each blood system:

- Single-dose status: Jk(a+b+), Fy(a+b+), and S+s+.
- Double-dose status for antigen: Jk(a+b–), Fy(a+b–), and S+s–.
- Double-dose status for antithetical antigen: Jk(a–b+), Fy(a–b+), and S–s+.

Because of the rare prevalence of the lack of all antigens [Jk(a–b–), Fy(a–b–), and S–s–] in white individuals, we excluded these antigen combinations. In each session, positive controls (in either double-dose or single-dose state) were tested. Saline solution was used as a negative control (i.e., blank) to test for possible contamination. Overall, 13 samples (including controls) were analyzed in each session in three ordered repetitions, over three equivalent sessions, performed on separate days. Positive and negative samples were arranged in an alternate sequence to limit the risk of carryover. In total, 702 tests were performed, including controls. Accuracy was evaluated on the 486 tests obtained from blood samples. The statistical analysis of reliability was performed on 324 tests, after excluding samples negative for the specific antigen (all negative samples returned negative tests), because their inclusion could deflate the rate of anomalous findings arbitrarily.

RBC Samples and Sera

RBC samples were obtained from leukoreduced packed RBC units resuspended in additive solution available in the blood bank inventory. A total of 14 units were selected from blood donors whose phenotype had been previously determined by test tube. Each RBC sample was used to test

one or more blood group antigens, depending on availability of sample units. Testing sessions were performed over 3 weeks. All samples were tested before the expiration date of the RBC units. Reagent RBC samples from identification panels Surgiscreen Resolve Panel A (Ortho Clinical Diagnostics), previously diluted up to a final concentration of 3 percent, were used as positive controls.

The ORTHO Sera (Ortho Clinical Diagnostics) are ready for use and contain sodium azide (concentration weight/volume <0.1%) as preservative and potentiators/bovine material. The ORTHO Sera characteristics are summarized in Table 1. The operating temperature ranged between 15°C and 30°C. The sera were stored between 2°C and 8°C if they could not be used within 8 hours. All reagents used are CE marked, conforming to the current European legislation.

Table 1. Technical description of the ORTHO Sera

Sera	Clonality	Method and cassette
Anti-Jk[a]	Human IgM monoclonal Ab (P3HT7 clone)	DAT using OCD reverse diluent cassette
Anti-Jk[b]	Human IgM monoclonal Ab (P3.143 clone)	DAT using OCD reverse diluent cassette
Anti-Fy[a]	Human IgG monoclonal Ab (DG-FYA-02 clone)	IAT using OCD IgG cassette
Anti-Fy[b]	Human polyclonal IgG Ab	IAT using OCD IgG cassette
Anti-S	Human IgG monoclonal Ab (P3S13JS123 clone)	IAT using OCD IgG cassette
Anti-s	Human IgG monoclonal Ab (P3YAN3 clone)	IAT using OCD IgG cassette

Ab = antibody; DAT = direct antiglobulin test; OCD = Ortho Clinical Diagnostics; IAT = indirect antiglobulin test.

Column Agglutination Technology and Its Interpretation

The ORTHO AutoVue Innova is an automated in vitro immunohematology analyzer that uses ORTHO BioVue System CAT with digital image processing. It basically integrates automation in multiple steps during the testing process, including pipetting, reagent handling, incubation, centrifugation, reaction grading, and interpretation by digital image processing and data management. In CAT, agglutinated RBCs are trapped above or in the column glass beads, whereas unagglutinated RBCs move through the column and form a pellet at the bottom.[15] A score of positive reactions can be assigned for CAT, with a maximum value of 4 when most of the RBCs migrate slightly below the reaction chamber and form a homogeneous front, and a minimum of 0.5 when few RBCs rise from the bottom typically going up just along one side of the column.[16] Conventionally, a score between 3 and 4 indicates a strong positive reaction, and a score between 0.5 and 2 defines a weak positive reaction. One of the following anomalous conditions may occur, however: mixed field (MF), fibrin (FIB), or an undefined score, essentially due to the following:

- MF: two distinct cell populations (i.e., agglutinated and unagglutinated cells) are present.
- FIB: presence of fibrin may generate a positive test result at the bottom of the reaction chamber.
- Undefined score: few agglutinates are generated, and analyzer reading is indefinite.

When anomalous data are generated, the analyzer may require manual confirmation of test results. In case of a discrepancy between the test result and the prior known phenotype, the donor's blood sample bag was retrieved and a new blood sample was sent to the reference laboratory of the blood transfusion service at the University Hospital A. Gemelli (Rome, Italy) for further serologic and molecular testing by sequence-specific primer (SSP) polymerase chain reaction (PCR).

Statistical Analysis

Descriptive tables were reported as absolute and relative frequencies. The accuracy of ORTHO Sera was evaluated by assessing sensitivity, specificity, and AUC, accounting for the clustering nature of repeated measures. A cross-classified mixed-effect logistic model was used for assessing the risk of measurement failure or misclassification as a measure of reliability. The model included only positive samples (i.e., samples that were single-dose or double-dose positive for the evaluated antigen). Replicates were hierarchically nested within cross-products of sessions and blood samples, representing a larger set of blood samples and sessions (i.e., the same blood sample would be used in multiple sessions, and the same session could include multiple samples). Therefore, both samples and sessions were treated as high-order (second) cross-level random effects. The antigen-specific combination (double-dose for a or b and single-dose [$c = \{a+, b+\}$]), the testing order of repetitions within each session (from 1 to 3), and the blood group system tested (Kidd, Duffy, MNS) each represented a fixed effect, which may not vary within each sample-session combination. Fixed effects may systematically contribute to the risk of measurement failure (misclassification) and explain part of the residual variability.

Null models were initially fit, which only included the random effects of sessions and samples, separately. Intraclass correlation coefficients were used for estimating the proportion of residual variance dependent on sessions or samples, respectively. Fixed effects were then added iteratively or alternatively to the models and were further expressed as odds ratios (ORs).

A p value <0.05 was considered statistically significant. The analysis was performed using Stata software, version 13.1 (StataCorp, College Station, TX).

Results

Accuracy of ORTHO Sera

Most of the ORTHO Sera displayed 100% sensitivity and specificity, except for anti-S (Table 2). This discrepancy was due to a single sample that yielded a false-negative result in one assessment with ORTHO Sera, but displayed a weak positive result in the remaining eight measurements. All ORTHO Sera displayed optimal AUC, equal to 1.00 in all cases except for anti-S (AUC 0.99).

Table 2. Sensitivity, specificity, and AUC of the ORTHO Sera

Sera	Sensitivity (%)	Specificity (%)	AUC
Anti-Jk^a	100	100	1.00
Anti-Jk^b	100	100	1.00
Anti-Fy^a	100	100	1.00
Anti-Fy^b	100	100	1.00
Anti-S	98	100	0.99
Anti-s	100	100	1.00

AUC = area under the receiver operating characteristics curve.

Reliability of ORTHO Sera

Reliability has been confirmed (both intra- and inter-assay). All repetitions gave the same intra-session and inter-session data, except for a single anti-S serum assessment. Nevertheless, manual interpretation of the reaction score was necessary in some cases. This need was attributable to the presence of some anomalous results, especially for expression of MF, FIB, or undefined score.

Table 3 presents the frequency of anomalous results (38 of 486 tests; 7.8%). Anomalous results only occurred in samples with any expression of antigens; those not expressing any antigen were always classified as negative by the analyzer, with no false-positive reactions. Manual interpretation was never necessary for results obtained with anti-Fy^b and anti-s sera, whereas data generated with anti-Jk^b needed manual interpretation in 14 of 81 cases (17.3%). Manual interpretation was necessary 15, 10, and 13 times in the first, second, and third repetitions, respectively (Table 4).

Table 3. Distribution of anomalous results by each individual ORTHO Sera reagent and type of anomaly

ORTHO Sera reagent	US	MF	FIB	Automated readings	Total
Anti-Jk^a	0	2	10	69	81
Anti-Jk^b	0	14	0	67	81
Anti-Fy^a	0	4	1	76	81
Anti-Fy^b	0	0	0	81	81
Anti-S	1	6	0	74	81
Anti-s	0	0	0	81	81
Total	1	26	11	448	486

US = undefined score: few agglutinates are generated; analyzer reading is indefinite; MF = two distinct cell populations (i.e., agglutinated and unagglutinated cells) are present; FIB = presence of fibrin may generate a positive test result at bottom of reaction chamber.

Table 4. Distribution of automated readings by order of repetition within sessions and reading output

Repetition	US/MF/FIB	Nil	Weak positive	Strong positive	Total
1	15	1*	4	88	108
2	10	0	4	94	108
3	13	0	3	92	108
Total	38	1*	11	274	324

Fisher's exact test: $p = 0.85$.

Table to include organic blood samples with reagent-specific positive status only.

*False-negative result with anti-S.

US = undefined score: few agglutinates are generated; analyzer reading is indefinite; MF = two distinct cell populations (i.e., agglutinated and unagglutinated cells) are present; FIB = presence of fibrin may generate a positive test result at bottom of reaction chamber.

In the mixed model, sessions and samples contributed to 38 and 18 percent, respectively, of the overall variance of measurement failure outputs. Table 5 shows absolute frequency distribution across replicates. The order of repetitions within sessions was not associated with the risk of anomalous findings (OR 0.89, 95% CI 0.55–1.43; $p = 0.62$). The risk of anomalous findings was then compared between blood group systems tested and according to the antigen-specific status (double dose versus single dose) of each reagent-sample combination across sessions. Compared with the Duffy system, assay reactions for the MNS system had an OR of 1.38 (95% CI 0.13–14.28; $p = 0.88$), and those for the Kidd system had an OR of 7.66 (95% CI 0.79–74.07; $p = 0.08$) for anomalous results. For single-dose samples, while also accounting for the blood system

Table 5. Distribution of automated analyzer readings by session and reading output

Session	US/MF/FIB	Nil	Weak positive	Strong positive	Total
1	3	0	1	32	36
2	1	0	3	32	36
3	1	0	0	35	36
4	12	0	0	24	36
5	14	0	0	22	36
6	0	0	0	36	36
7	4	0	2	30	36
8	3	1*	2	30	36
9	0	0	3	33	36
Total	38	1*	11	274	324

Fisher's exact test for the equality of the proportions of UF/MF/FIB to Nil outputs over the row totals, across sessions: $p < 0.001$.

Table to include organic blood samples with reagent-specific positive status only.

*False-negative result with anti-S.

US = undefined score: few agglutinates are generated; analyzer reading is indefinite; MF = two distinct cell populations (i.e., agglutinated and unagglutinated cells) are present; FIB = presence of fibrin may generate a positive test result at bottom of reaction chamber.

tested, anomalous results displayed an OR of 1.24 (95% CI 0.39–4.00; $p = 0.71$) with double-dose samples (under the assumption of constant effect between a and b double-dose statuses). Sensitivity analyses compared the Jk effect with the effect of Fy and Ss, which were assumed constant. Results were similar (OR 6.49, 95% CI 0.93–45.28; $p = 0.06$).

Misclassification of ORTHO Sera with Potential Antigenic Variants

A critical sample was used in our validation plan for testing both the MNS and Kidd systems. The donor was a white man, whose RBCs were known to be group O, Rh phenotype of DCe/cde, Jk(a–b+), and S+s+. During the assessment with ORTHO Sera anti-S, one single false-negative result was observed, and we found one single undefined scoring and scores of 0.5 in the remaining tests. The anti-s test gave no errors.

Assuming a possible qualitative or quantitative antigenic variant, we shipped a new sample to a reference laboratory. The sample was analyzed with both serologic typing (NEO Immucor automated system [Immucor, Norcross, GA]) and PCR-SSP (RBC-Ready Gene MNS kits [inno-train Diagnostik GmbH, Kronberg, Germany]). The tests yielded discordant results. More specifically, a strong positive score was found by assessing S antigen with serologic testing, whereas molecular biology testing was negative for the S allele.

Discussion

Our institution decided to implement an automated method for serologic typing in the local laboratory because of the increasing burden of multiple alloimmunizations against common RBC antigens in transfusion practice.[5,7] It may be quite challenging to find donors for patients with complex alloimmunization,[2] and the availability of many extensively typed donors would allow us to more easily and rapidly identify compatible RBC units for these patients.[17,18]

The current Italian recommendations suggest that the activity carried out in blood transfusion services must follow a validation and qualification process.[14] In immunohematology, the purpose of the validation process is to ensure accuracy and reliability of tests. In our study, we performed a prospective method validation. After the first phase (IQ and OQ), we focused on PQ, using an experimental design entailing testing each factor in intra-series and inter-series triplicates, thus ensuring repeatability and reproducibility of the assay. The very stringent primary objective for accuracy (sensitivity and specificity of 100%) was accomplished for all tests except for anti-S serum, for which the sensitivity was 98%. Consistently, the use of the AUC, an additional index of accuracy, which combines both sensitivity and specificity, showed the maximum score of 1 for all sera, with the only exception of anti-S (AUC 0.99). Similar findings emerged from reliability analyses (i.e., analyzing both repeatability and reproducibility), in which results of all sessions were optimal for all sera, except for anti-S.

A weak score was generated in the critical sample over multiple anti-S assessments, so that further scrutiny was necessary. Molecular genotyping can support serologic testing. High-efficiency automated platforms currently allow us to extensively genotype donors and patients in blood banks or in the reference immunohematology laboratory.[17,18] Genotyping could also predict weak or variant alleles, some of which may still generate challenges for the correct assessment of antigenic expression.[19] In the suspicion of a possible S antigen variant, we consulted a reference laboratory for further serologic and molecular testing by PCR-SSP. These assays generated discordant results, because the serologic assessment with automated liquid phase was positive, whereas failure of gene amplification with selected primers was observed using molecular biology. The MNS system has many rare phenotypes, characterized by specific antigenic expression patterns—the best known is the Miltenberger series.[19] Discrepancy between serologic and molecular testing was suggestive for the presence

of a *GYPB* gene polymorphism close to the region of the PCR primer, which ultimately prevented in vitro amplification. A possible explanation may be the presence of a hybrid *GYPA-GYPB* gene, secondary to unequal gene crossing-over, thus causing partial expression of S from the chimeric gene in the absence of the target region of *GYPB* primer. This hypothesis could only be confirmed by gene sequencing, however, which was not accessible at the time of this study. This evidence suggests that weak or variable hemagglutination requires molecular diagnostic investigation that can define a specific gene polymorphism using commercial validated in vitro diagnostic reagents; on the other hand, this approach may result in discovery of new undefined polymorphisms requiring gene sequencing for a complete definition. Further studies may be needed to define whether or not the combination with genotyping may be superior to hemagglutination, which is still widely considered the conventional serologic method.

Because of the observation of this false-negative result, some doubts were raised as to whether the validation of anti-S serum could be considered successful. As a potential solution to this issue, the Italian recommendations may be helpful, especially for managing the worst cases.[14] Critical factors may sometimes emerge in routine activity and are now regarded as worst cases. According to the results of our validation, it can be hypothesized that an antigenic variant may be seen as a paradigmatic worst case. The recommendations clearly state that a worst-case test does not necessarily overthrow a process of validation because of the specific conditions under which validation is carried out, and that are clearly different from routine activity. It can be concluded that our validation was successful even for the anti-S serum.

Although in most cases the hemagglutination procedures are manually performed via test tube, this process has advantages and drawbacks. The incorporation of blood bank automation systems can help reduce human errors, improve standardization, alleviate heavy workload, and improve turnaround time.[9–11] From occupational safety and health perspectives, biological safety of operators can also be improved by adopting automated systems. The minimal sample handling procedures reduce operator exposure to potentially hazardous biological materials. Moreover, a traceable database of laboratory records can be dynamically and cumulatively populated, since the image captured during interpretation of reactions can be permanently archived.[11] Result interpretation may also be automated, whereas the manual assay still needs operator reading, which is inherently associated with subjective evaluation and broad interobserver variability.[10] Nevertheless, ORTHO Sera sometimes needed manual interpretation for detecting anomalous results in our analytical evaluation, and this may lead to wastage of time, thus affecting work organization. Overall, anomalous findings could be observed in 38 of 486 (7.8%) tests, especially for MF (26 cases, 14 with anti-Jk^b) and FIB (11 cases, 10 with anti-Jk^a). Multiple factors could theoretically explain the risk of obtaining anomalous results. Among the fixed conditions, the most obvious include the different performance of sera and the expression of blood group system antigens. The order of the three different intra-session repetitions could also have an impact on analyzer or reagent performance. To evaluate randomly acting or systematic factors involved in the generation of anomalous findings also affecting reliability, we used a cross-classified mixed-effect logistic model. This model is intended to capture whether random effects should be considered in the analysis due, for example, to the unobserved randomly variable state of preservation and/or handling of RBC units (sample effect) or to non-controllable and largely unpredictable external conditions under which the session has been carried out, such as environmental temperature, operator's accuracy, analyzer functionality, and so forth (i.e., session effect). Notably, none of the fixed factors, including order of replications, blood system tested, or antigen combination, was significantly associated with anomalous data.

The comparison of blood group systems needs further scrutiny. Compared with the reference Duffy system, no differences could be appreciated with MNS, whereas the Kidd system had some evidence, albeit statistically weak, of generating a larger number of anomalous results, both when compared to the other systems as separate entities, or as uniform entities. From a speculative perspective, the different behavior of the Kidd system antigens may be attributable to the fact that anti-Jk sera are monoclonal IgM antibodies that, unlike ORTHO Sera, do not necessitate anti-human globulin addition using reverse cassettes. Random factors may determine variability of anomalous findings, namely, the selection of blood samples (as RBC units) and the analytical performance of the session. Both factors were found to be associated with a substantial part of the risk of anomalous findings ($p < 0.01$), contributing 18% (intra-class correlation coefficient) and 38% to the overall variability, respectively. Taken together, the results of our analysis suggest that the frequency of anomalous results is in large part attributable to random (uncontrollable) factors. Although there is no immediate solution to reduce this component of variability, additional studies may be warranted for investigating specific

environmental factors that more likely may influence the random variability attributable to samples and sessions.

A major strength of our study is that we first carried out an experimental validation in an Italian transfusion center shortly after publication of the Italian recommendations.[14] Validation studies are routinely carried out by pharmaceutical and plasma-derivation industries, and the current recommendations actually suggest the use of an experimental design borrowed from the routine.[14] The universally agreed-upon criterion of "exhaustive control" is currently represented by testing three consecutive batches, which is also regarded as the minimum requirement for process validation. Not fewer than three measurements for each of the identified stratification factors should be carried out, and we exactly matched this strategy in our evaluation.

The limited number of analyzed events should instead be considered a limitation of our study, especially for conducting the multivariable reliability analysis. This result is obviously attributable to the experimental design, which was based on the minimum number of samples required for validation, but also to reasonable cost restrictions for the availability and use of samples with a known phenotype.

Conclusions

In conclusion, we successfully carried out the validation of an automated CAT RBC typing method with Ortho Clinical Diagnostics' ORTHO Sera against antigens in the Kidd, Duffy, and MNS blood groups systems. These sera displayed high accuracy and reliability and permit a viable automated procedure for rapid phenotyping of large numbers of donors.

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