An overview of the Progenika ID CORE XT: an automated genotyping platform based on a fluidic microarray system

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Automated testing platforms facilitate the introduction of red cell genotyping of patients and blood donors. Fluidic microarray systems, such as Luminex XMAP (Austin, TX), are used in many clinical applications, including HLA and HPA typing. The Progenika ID CORE XT (Progenika Biopharma-Grifols, Bizkaia, Spain) uses this platform to analyze 29 polymorphisms determining 37 antigens in 10 blood group systems. Once DNA has been extracted, processing time is approximately 4 hours. The system is highly automated and includes integrated analysis software that produces a file and a report with genotype and predicted phenotype results. ***Immunohematology* 2015;31:62–68.**

Key Words: blood group, DNA-based typing methodology, genotyping

The Progenika ID CORE XT (Progenika Biopharma-Grifols, Bizkaia, Spain) is a highly automated testing platform based on the Luminex XMAP (Austin, TX) testing platform.[1–4] The Luminex system is a modified flow cytometer that utilizes probes attached to microspheres and (red and green) lasers to quantify the amount of a given analyte that has attached to a specific probe. The microspheres are labeled with different ratios of two red fluorochromes, creating 100 distinctive bead populations or "spectral addresses." The Luminex system has a wide spectrum of applications requiring high-throughput detection of multiple nucleic acid sequences in a single reaction (multiplexing), including HLA and HPA typing, which are potentially of interest in blood centers and transfusion services.[1] For this reason, Luminex instruments are already in place in many laboratories, facilitating the implementation and routine use of the ID CORE XT blood group genotyping system.

ID CORE XT analyzes 29 polymorphisms determining 37 antigens of the Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright, and Lutheran blood groups, shown in Table 1. The less common RhCE alleles assayed are shown in Table 2. This is a second-generation assay, replacing the ID Core+. In the ID CORE XT system, targeted regions of genomic DNA from 10 different red cell blood group systems are amplified in a multiplex polymerase chain reaction (PCR) using biotinylated dCTP. The amplification products are then hybridized onto oligonucleotide probes attached to the microspheres and labeled with fluorescence-conjugated streptavidin. When the beads are analyzed with the Luminex system, the presence of the specific polymorphism is determined by the correlation of the fluorescence signal intrinsic to each microsphere in the red region of the spectrum, with the presence or absence of a corresponding fluorescent signal in the green region of the spectrum. An overview of the system is shown in Figure 1. The system is highly automated, and includes integrated analysis software that produces a file with the genotype results and the corresponding predicted phenotype. The software helps track samples and reagents through the system.

Table 1. Red cell antigen typing using ID CORE XT

Blood group	Variants and ISBT number
Rh	C(RH2), E(RH3),c(RH4), e(RH5), CW(RH8), V(RH10), hr^S(RH19), VS(RH20), hr^B(RH31)
Kell	K(KEL1), k(KEL2), Kp^a(KEL3), Kp^b(KEL4), Js^a(KEL6), Js^b(KEL7)
Kidd	Jk^a(JK1), Jk^b(JK2), JKB_null(IVS5-1a), JKB_null(871C)
Duffy	Fy^a(FY1), Fy^b(FY2), FYB_GATA, FYB[265T]_FYX
MNS	M(MNS1), N(MNS2), S(MNS3), s(MNS4), U(MNS5), Mia(MNS7)
Diego	Di^a(DI1), Di^b(DI2)
Dombrock	Do^a(DO1), Do^b(DO2), Hy(DO4), Jo^a(DO5)
Colton	Co^a(CO1), Co^b(CO2)
Cartwright	Yt^a(YT1), Yt^b(YT2)
Lutheran	Lu^a(LU1), Lu^b(LU2)

Materials and Methods

Microscopic spherical particles with a 5.6-micron diameter, called microspheres, serve as a solid phase for molecular detection. The microspheres are internally dyed with two spectrally distinct fluorochromes, red and infrared,

Table 2. Less common RhCE alleles assayed, ID CORE XT

Allele (ISBT)	Allele (ID CORE XT)	RHCE sequences that differ from consensus, with underline indicating interrogated positions
*RHCE*ceAR*	*RHCE*ceAR*	c.48C, c.712G, c.733G, c.787G, c.800A, c.916G
*RHCE*CeFV*	*RHCE*CeFV*	c.667T, c.697G, c.712G
*RHCE*CeVG*	*RHCE*CeVG*	c.712G, c.733G, c.787G
*RHCE*cEFM*	*RHCE*cEFM*	c.697G, c.712G
*RHCE*ce712G*	*RHCE*ce[712G]*	c.712G
*RHCE*ce733G*	*RHCE*ce[733G]*	c.733G
*RHCE*ce733G,1006T*	*RHCE*ce[733G,1006T]*	c.733G, c.1006T
*RHCE*CE-D(-)-CE (various alleles)*	*RHCE*D(2,5,7)-CE*	c.307, c.676, c.712, c.733, c.1006[1]
*RHCE*cE697G,712G,733G*	*RHCE*cE[697G,712G,733G]*	c.697G, c.712G, c.733G
*RHD*DIIIa-CE(3-7)-D RHCE*ce48C,733G,1006T*	*RHD*r'S - RHCE*ce[733G,1006T]*	IVS3+3100g, c.733G, c.1006T

[1]Absence of signal for these probe sets.

which emit light in different regions of the optical spectrum. The combination of the dyes in different concentrations results in 100 different fluorescent color tones of red and infrared, each color having a unique spectral address defining a bead class. Different allele-specific oligonucleotide probes are coupled to the microspheres of each bead class. After DNA extraction,

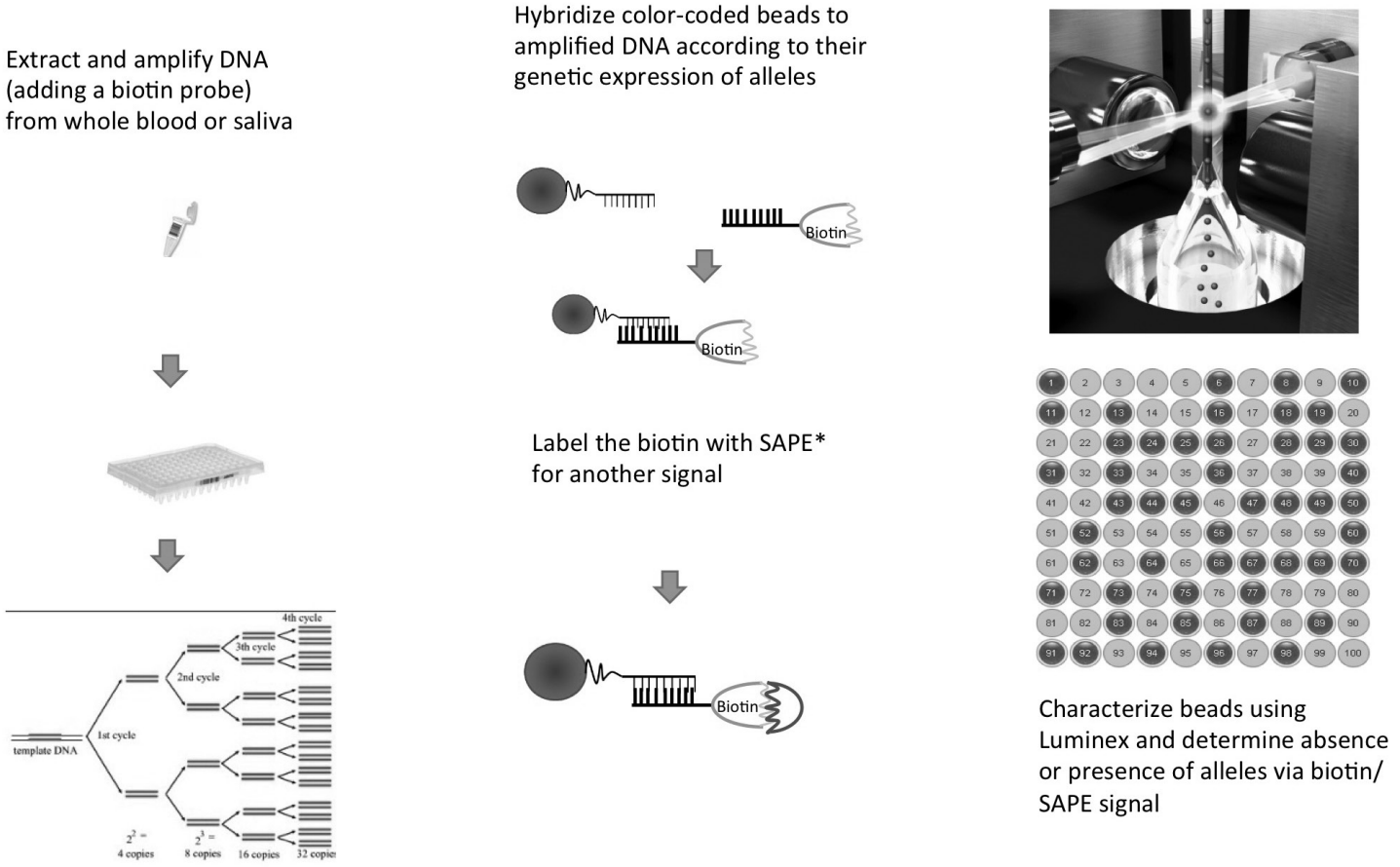

Fig. 1. Progenika ID CORE XT method. SAPE = streptavidin-phycoerythrin.

amplification is performed with biotin-labeled nucleotides. During hybridization, the amplified sequences bind to the oligonucleotide probes. Streptavidin-phycoerythrin (SAPE) is used as the labeling molecule that will then bind to the biotin. Quantification of each bead class population is performed using the Luminex modified flow cytometer. The red laser sorts the beads into their different classes, while the green laser excites SAPE. The software counts each SAPE signal and associates it to the unique bead class.

The ID CORE XT is considerably simplified compared with the early ID CORE+ testing system. An overview of the workflow is shown in Figure 2.

The main steps in the procedure (Fig. 2) include:

1. **DNA extraction.** DNA extraction is performed from EDTA whole blood. A total of 100 ng DNA (5 µL) is required per sample. DNA concentrations between 20 and 80 ng/µL are recommended in the ID CORE XT package insert. Nevertheless, DNA concentrations between 12 and 150 ng/µL were tested during beta-testing evaluations and gave satisfactory results. The genomic DNA purity (A260/A280 ratio) should be in the 1.63 to 2.1 range, according to the manufacturer. Although DNA concentration can be measured by a spectrophotometer, in practice, we have not found this necessary.
2. **Amplification.** The amplification process, set up in the pre-PCR area, includes preparing a PCR mix by adding the HotStarTaq (Qiagen, Venlo, the Netherlands) polymerase (2.5 U/reaction) to the ID CORE XT PCR "master mix," containing the primers, biotinylated nucleotides, and buffer solution together. A total of 20 µL of this mixture is then added to the PCR plate for each sample and control, followed by 100 ng of each DNA sample in a volume of 5 µL. The plates are sealed with an adhesive PCR plate film, centrifuged briefly to collect the liquid at the bottom of the wells, and placed in the thermal cycler in the post-PCR area. Amplification requires 2 hours, 40 minutes.
3. **Hybridization and labeling.** After vigorous mixing, 46 µL of the "beads master mix" is dispensed into each well of a Costar plate (Bio-Rad Laboratories, Hercules, CA). For each sample, 4 µL of the PCR product is added to each well. The plate is covered and placed in the thermal cycler at 52°C for 30 minutes. SAPE in a dilution buffer is then added to each well. After labeling with SAPE, the samples should be analyzed immediately in the Luminex.
4. **Quantification.** Quantification of the relative amounts of labeled PCR product hybridizing to each

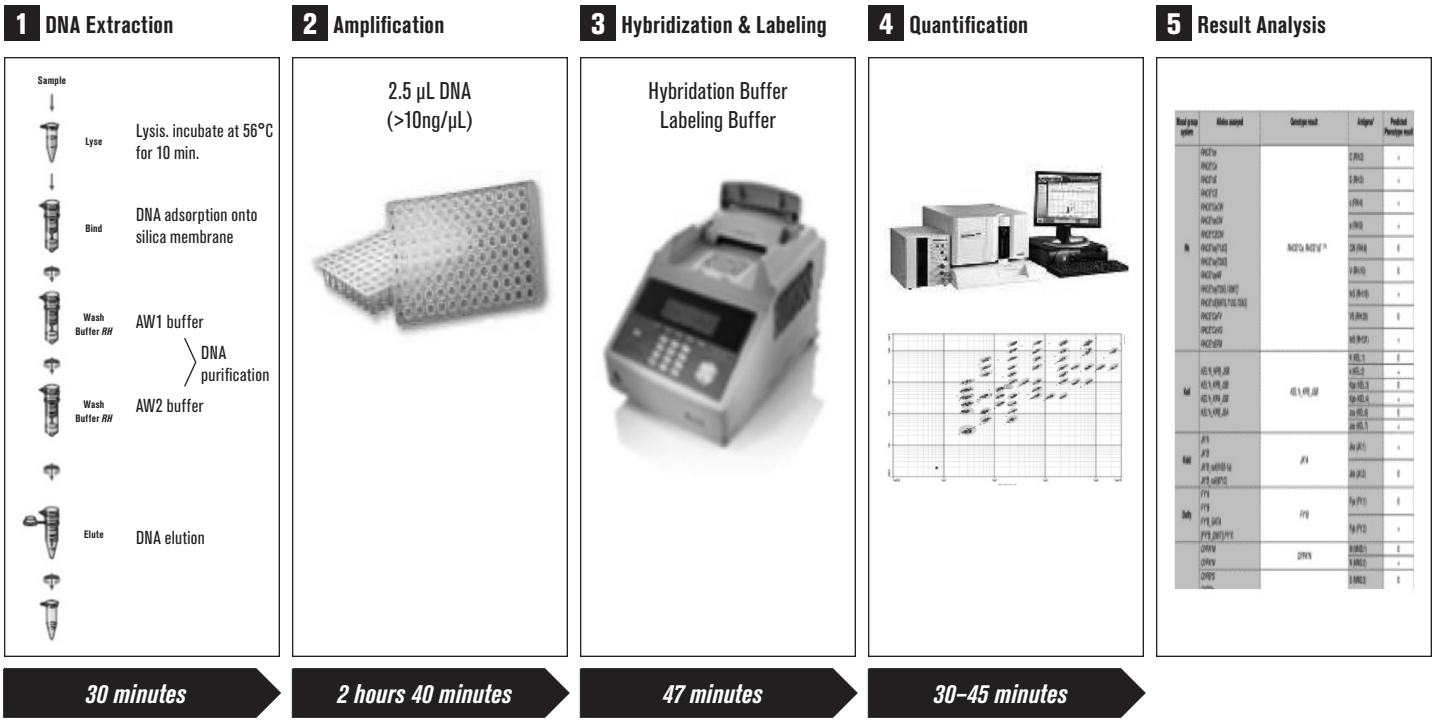

Fig. 2. Progenika ID CORE XT workflow.

microsphere is fully automated and takes 30–45 minutes.

5. **Results analysis.** The analysis software automatically imports the quantification data from the Luminex and produces a report with the genotype and predicted phenotype. Footnotes are used for possible alternate genotypes or for phenotypes predicted to have weak, partial, or variable antigen expression. For example, a genotype interpreted as *RHCE*Ce, RHCE*cE,* may have a footnote "also possible although less likely: *RHCE*ce, RHCE*CE*." A homozygous *RHCE*ce* (733G) genotype will have a predicted c+ phenotype with the footnote "detected as partial by some reagents" and e+ phenotype with the footnote "detected as weak or partial by some reagents."

The instrumentation needed includes:

1. Thermal cycler: The ID CORE XT system has been validated for both the gold-plated and the aluminum-plated 96-Well Geneamp PCR System 9700 (Thermo Fisher Scientific, Waltham, MA), and for the Veriti or Veriti Dx 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA).
2. Luminex LX 100 or LX 200 instrument with the Luminex 100 IS or xPONENT software, respectively.
3. Non-refrigerated microcentrifuge.

Strengths and Limitations

Ease of Use

The system can test from 6 to 94 samples, with two controls. The flexibility in the number of samples is useful, permitting more urgent diagnostic testing on relatively small numbers of samples as well as donor testing on larger sample batches. As detailed in Materials and Methods, the amplification and hybridization steps involve little pipetting, since reagents have been combined in "master mixes." Once the samples are entered into the Luminex, the system is totally automated. The overall time to analyze the samples, once the DNA has been extracted, is approximately 4 hours. Because the system is automated and uses software to interpret the results, little actual expertise in the molecular biology of the red cell antigen systems is required, although the possibility of visualizing the raw data graphs may provide some insight in cases of discordances between phenotyping and genotyping or "No Call" results. The software is common to other BLOODchip ID XT (Progenika Biopharma-Grifols) genotyping systems, such as the ID HPA XT, which allows simultaneous sample processing and data analysis in hybrid batches.

In summary, the procedure is rapid and simple, requiring less hands-on time and a faster turn-around time than the earlier version of the system. The speed and flexibility make it optimal for analyzing both patient and donor samples. Limitations to the system include the impossibility to reread a sample and the separate acquisition of the plates used for amplification and hybridization.

Data Handling

Data from the Luminex can be uploaded into a laboratory information system and formatted for XML, ASTM, or HL7 files. This is a major advantage, particularly in the testing of donor samples. Currently, at Canadian Blood Services (CBS), we are developing a database containing the results of donor testing, to allow donors with given genotypes to be easily searchable. Once the technology is licensed in Canada, we would like to export these results into our eProgesa operating system. Similarly, different blood centers in Europe are also adapting the communication protocol between the XT software and their laboratory information systems to automatically upload results into their blood center donor database. The immunohematology reference laboratory of the Blood and Tissue Bank in Barcelona is also adapting the automatic transfer of patient blood group genotype results to the laboratory information system, avoiding the risk of manual transcription errors.

Systems Interrogated

ID CORE XT tests for the presence of 29 polymorphisms in 10 blood group systems (Table 1). The system is closed, in that laboratory users cannot add polymorphism content. The xMAP technology is open, however, and this makes upgrading the system possible by the manufacturer in future versions. A major improvement over the earlier version of the test is the detection of more RhCE variants: the low-incidence antigens V (RH:10) and VS (RH:20) and the high-incidence antigens hr^S (RH:19) and hr^B (RH:31) (Table 2). These antigens are of great interest in the predominantly black patient population with sickle cell disease. Investigation and transfusion support of these patients is an important component of the service provided by our reference laboratories. Challenges related to these complex antigens are outlined in the following section. Other advantages are inclusion of the *GYPB*Mur, YT*A/YT*B,* and some *JK* null alleles into the platform. The *LU*A/LU*B* system alleles have also been added in this version of the system. The inclusion of low-incidence polymorphisms is useful

for the reference laboratory, because it permits elimination of the possibility of rare specificities in alloimmunized patients. Discrepant results between serology and genotyping can occur because of an allele drop-out in assays using primer elongation, but the probe hybridization-based ID CORE assay is not affected.

Limitations include the absence of the *SC*1/SC*2* and the *LW*A/LW*B* alleles. The system does not include RhD, or the ABO system. The system also does not provide information on zygosity or haplotype determination. As with any system, not all rare polymorphisms are interrogated; therefore, there may be discordances between genotyping and phenotyping results.

Challenges Related to Complex Rh Antigens

One limitation of targeted genotyping platforms derives from the restricted number of relevant polymorphic positions that they interrogate because of "space" constraints. This limitation becomes more evident for high-incidence antigens, such as hr^B and hr^S, for which expression can be affected by genetic variants at multiple positions. In particular, hr^B and hr^S prediction is based on the sequence at polymorphic positions c.676, c.712, and c.733, although their expression can also be affected by variant sequence at c.254, c.538, and c.667. ID CORE XT predicts V and VS antigens based on the sequence at three polymorphic positions, namely, c.712, c.733, and c.1006. Although c.733 is the key position, the sequences at c.712 and c.1006 can modulate its effect, as is the case for *RHCE*ceAR* and *RHCE*ce48C,733G,1006T* alleles, respectively. The effect of the sequence at c.733 on V and VS can also be modulated by variant sequence at c.340, found in the *RHCE*ce340T, 733G (RHCE*ceJAL),* allele. This polymorphic position is not interrogated by ID CORE XT, although the variant frequency is significantly lower than that at positions c.733 or c.1006.

The accuracy of phenotype prediction for these complex antigens is inversely proportional to the population frequency of uninterrogated variants. Although this may appear as a critical limitation, only a portion of genotype miscalls translate into erroneous phenotype predictions. Genotyping platforms have implemented various ways to mitigate or avoid the consequences of such limitation. First, interrogated positions are selected on the basis of their relevance (i.e., their effect on antigen expression) and their population frequency, where the latter may refer to the global population or to a specific race. Second, the data analysis software is designed to make default calls for the more likely genotypes, with likelihood being a function of variant and haplotype frequencies. Third, nomenclature that is inclusive of all the alleles that might explain the variant sequences identified is used. Finally, as mentioned in Materials and Methods, footnotes are included in the report to inform the user of alternative interpretations of the data (other possible genotypes and predicted phenotypes).

Licensure

The system is currently CE-IVD marked since June 2014, but as of September 2015 has not yet received licensure by the U.S. Food and Drug Administration or by Health Canada.

Results

Validation and Experience with the Method

The ID CORE XT assay was initially evaluated in a small-scale pilot study undertaken by Progenika in Spain, in which 100 genomic DNA samples, previously typed by BLOODchip Reference v.4.0, ID CORE+, and sequencing, were tested.[5] The samples included 57 patients and 43 donors of both white and African descent. The genotype results were 100 percent concordant with the reference methods. A second validation study performed by Progenika on 42 commercial cell line DNA samples of diverse ethnic origin demonstrated 100 percent concordance with BLOODchip reference v4.0 and sequencing data.[6]

A performance evaluation study using 86 blood donor samples was then carried out at an alpha site (Sanquin Blood Supply Foundation, Amsterdam, the Netherlands) with the aim to test the ID CORE XT assay and the proprietary BIDS XT analysis software with samples previously typed serologically.[7] Although serology was considered the reference method, the samples were also genotyped, when possible, by a multiplex ligation-probe amplification (MLPA) molecular method developed in-house. The ID CORE XT and the BIDS XT software performed correctly in a wide range (60–1000 ng) of total DNA/assay and in different sample batch sizes. The five discrepancies encountered with serologic results were resolved by MLPA and sequencing, which showed results concordant with ID CORE XT.

A clinical validation with 1000 samples was then carried out at two different sites. The samples were selected following the common technical specifications established for blood group reagents in Appendix II of European Directive 98/79/CE. ID CORE XT results for C, c, E, e, K, Kidd, and Duffy antigens were compared with results obtained by CE-marked serology typing reagents. The remaining antigens (which belong to self-certification) were evaluated against serology data when commercial antibodies were available or otherwise against well-established molecular reference methods (either

bidirectional DNA sequencing or BLOODchip Reference). The ID CORE XT genotyping results of 100 of the 1000 random samples, were additionally compared with results from bidirectional DNA sequencing or BLOODchip Reference. The high sensitivity and specificity rates obtained for all tested antigens highlighted the reliability and accuracy of ID CORE XT genotyping.

The ID CORE XT was validated in Brazil using a total of 229 donor and patient samples previously genotyped using in-house genotyping methods or the HEA BeadChip™ (Bioarray, Warren, NJ).[8] A reproducibility rate of 100 percent, repetition rate of 0 percent, and a "No Call" or invalid result rate of 1.7 percent were observed. Results were concordant with previous genotyping in 223 out of 229 (97.4%) samples. Four of the six discordant samples involved the low-incidence RhCE variants V (RH:10) and VS (RH:20). In three of these samples, genomic sequencing results were concordant with the ID CORE XT, and, in one sample, results were concordant with the HEA. One sample was identified as possible r′S by HEA, but was not confirmed by ID CORE XT, Beadchip RhD and RhCE kits, or sequencing. ID CORE XT uses the IVS3+3100a>g SNP present in all type I r′^{S} variants to accurately predict this phenotype. Finally, one sample was discrepant for K, with investigation by serology and sequencing showing a false-positive result in the ID CORE XT.

In a recent international, multicenter study performed at three laboratories in Italy, Spain, and the UK, the performance in real-life conditions of the ID CORE XT were compared with the current methods of the participating centers. Excellent results of concordance and processing time were obtained in the more than 250 samples tested, which included a number of low-incidence rare variants. Publication of the complete results of both the clinical validation and the multicenter study is in progress (personal communication, N. Nogues). A small validation study of 47 hematology patients was also carried out in the UK, with one discrepant M typing under investigation.[9]

Current organizations using this method include CBS and Héma-Québec in Canada and Carter BloodCare, Gulf Coast Regional Blood Center, Lifeshare Blood Centers, and ITxM/Virginia Blood in the United States. Over 27,000 samples have been tested internationally using ID CORE XT, including several hundred samples in Barcelona and at CBS. In Brazil, in-house validation is currently being performed at the Molecular Immunohematology Laboratory, Hemocentro-State University of Campinas, Sao Paulo. Clinical applications include testing of selected donors (e.g., donors who may be U– or U+^{var} on serologic testing), and investigation of difficult patient samples (e.g., patients with autoantibodies). The number of alleles interrogated, particularly in the Rh system, assists in genotyping patients with sickle cell disease and black donors who might express similar variant alleles.

Conclusion

In conclusion, the Progenika ID CORE XT is a highly automated testing platform, permitting testing on small or larger sample batches. Implementation is relatively easy, particularly because the Luminex platform is already in use in many immunohematology laboratories. Testing is rapid and simple, with integrated analysis and report generation. As with any genotyping platform, not all alleles are probed by the system, and results must be interpreted considering the clinical context and serologic findings.

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