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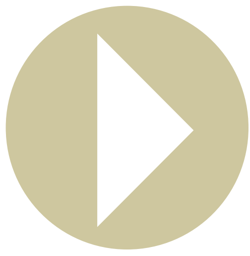

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EVALUATION OF THE NEO AUTOMATED BLOOD BANK SYSTEM FOR BLOOD TYPING AND ANTIBODY SCREENING AND IDENTIFICATION

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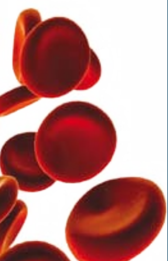
BACKGROUND: The automation of the blood bank technologies is a need to decrease the human error and increase the efficiency of human resources.
The validation is a complex process. The objective of validation is to produce documented evidence that provides a high level of assurance that all parts related to the use of an automated system will work correctly and consistently.

AIM: In order to replace in routine the Equipment Galileo System, we implemented the process of validation of the equipment NEO.

METHODS: With the supplier we organize a validation plan that includes:
311 randomly selected samples from routine patients and donors were tested for ABO/D blood grouping (forward and reverse) grouping (ABOD full- anti-A, Anti-B, - AB; Rh Control, D Rapid, D Novaclone, A1 & B cells).
436 randomly selected samples from routine patients and donors were tested for Rh phenotyping, Kell antigen (Rh Control, Anti-C, Anti-c, Anti-E, Anti-e, Anti-K).
122 randomly selected samples from routine patient and donors were tested antibody screening (2 cells).
The analytical performance of the NEO has been established by in-house testing Galileo. The evaluation was performed comparing the results obtained with both equipments. The results from both methods were assembled as paired data (NEO vs Galileo) .The performance of each method/test has been summarized as follows: All results were compared (after discrepancies have been investigated); % NTD, % Equivocal and Error rates for each method have been calculated. Each valid result of the Antibody Screening has been allocated as True Negative (TN), True Positive (TP), False Negative (FN) (negative result with a clinically significant antibody) or False Positive (FP). The calculation of the reactive rate, sensitivity, specificity, positive predictive value and negative predictive value of each method has been done according to the formulae: Reactive Rate of Test Method=((TP+FP)/ Nº sample tested) x100; Specificity of test Method=(TN/(TN+FP))x100; Sensitivity of the Method=(TP/(TP+FN))x100; Positive Predictive Value of Method= (TP/(TP+FP)) x100; Negative Predictive Value of the Method = (TN/(TN+FN))x100.



We also evaluate the time of all the procedures in both equipments.

Table 1- Evaluation values.

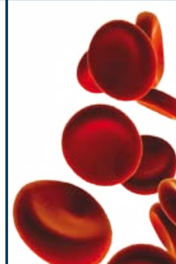
	ABO/D blood grouping		Rh/K phenotyping		AntibodyScreening	
	NEO	**Galileo**	**NEO**	**Galileo**	**NEO**	**Galileo**
Number of Samples Tested	311		436		122	
True Positives					2	2
True Negatives					119	120
False Positives					1**	0
False Negatives					0	0
% NTD	4,2*	4,5	0,2	0,2		
Error Rates	0	1	0	0		
Reactive Rate of Test Method					1,6%	1,6%
Specificity of Test Method					100%	100%
Sensitivity of Test Method					100%	100%
Positive Predictive Value of Method					75%	100%
Negative Predictive Value of Method					100%	100%

*One sample mixed field type confirmed.
** This sample was confirmed to be positive enzyme- only.

Table 2 - Testing Times

	ABO/D blood grouping		Rh/K phenotyping		AntibodyScreening	
	NEO	**Galileo**	**NEO**	**Galileo**	**NEO**	**Galileo**
Testing Time (min)	450 reactions/hour	450 reactions/hour	450 reactions/hour	450 reactions/hour	30 min	40 min

CONCLUSIONS: This study demonstrated that the NEO system has a similar performance when compared with Galileo which allow us to implement the equipment in our routine. As an observational note NEO allows to identify mixed field type reactions. From the logistical point of view this equipment improves the work flow of the laboratory routine.



EVALUATION OF NEO IMMUCOR FULLY AUTOMATED SYSTEM FOR DONOR BLOOD GROUPING

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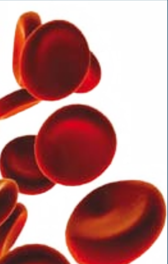
BACKGROUND: For its immune-hematology laboratory, the Transfusion center of the University Medical Center of the Johannes Gutenberg University Mainz, Germany decided to automate the donor blood grouping by replacing the current manual methods (DiaMed ID-Micro Typing System) with a fully automated system (Neo Immucor®).

AIMS: In order to evaluate the Neo's performance, we tested it in parallel with the manual methods. We have tested 683 first time donors for ABO forward and reverse grouping, Rhesus (C,Cw,c,D,E,e) and Kell phenotyping. The A blood group donors (330) were tested for A subgroups. The Kell positive donors (62) were tested for Cellano antigen. The Rhesus negative donors (123) were tested for Weak D type. 991 foreknown donors were tested for ABD forward grouping and 283 foreknown donors for antibody screening.

METHODS: The first time donors were tested with two manual methods: in plates and gel cards. The blood group screening for the foreknown donors was performed in tubes and the antibody screening in gel cards. The system used with the manual method was DiaMed ID-Micro Typing System. Neo performed the tests in microplates. The ABO forward and reverse grouping and Rhesus phenotyping tests were based on the agglutination principle. The A subgrouping, Rhesus, Kell and Cellano phenotyping, Weak D typing and antibody screening were based on Capture technology (solid phase adherence). The antibody screening was performed with a pooled cell.

RESULTS: The evaluation of the first time donors for ABO forward and reverse grouping, Rhesus and Kell phenotyping found 1,68% of the tests (23 of 1366) uninterpretable by Neo due to weak reaction with different reagents. 9 samples (O blood group donors) of the 23 reacted weakly with A1 test erythrocytes. The A subgroup evaluation found 7,27% (24 samples of 330) samples uninterpretable by Neo. The routine verification of the manual method found 3 interpretation failures. The Cellano evaluation found one discrepancy between Neo's capture technology (positive reaction) and the two manual methods (both reacted negative). The PCR confirmed Neo's result. Apart from that, the results were consistent. The evaluation of the ABD forward grouping found 0,5% of the tests (5 samples of 991) uninterpretable by Neo and one Rhesus D inconsistency, due most likely to human failure. Neo's result was consistent with the known donor's blood group. By the antibody screening two samples tested positive with Neo's capture technology and negative with the manual method. The discrepant samples tested negative with enzyme ID-Card.

SUMMARY/CONCLUSIONS: The Neo Immucor® automated readings of test reactions were consistent with the manual method's readings. Some tests were uninterpretable by Neo due to low reaction intensity by difficult samples and required visual reading by the laboratory technician. In the Cellano phenotyping the Neo Immucor® capture test showed a higher sensitivity. The automated antibody screening showed a slightly lower specificity with no clinical consequence. In conclusion, the fully automated system (Neo Immucor®) allows a safe determination of donor blood groups and reduces the possibility of human failure. Furthermore, it reduces human labor and turnaround time.



USE OF GENOTYPING IN NEONATAL ALLOIMMUNE THROMBOCYTOPENIA

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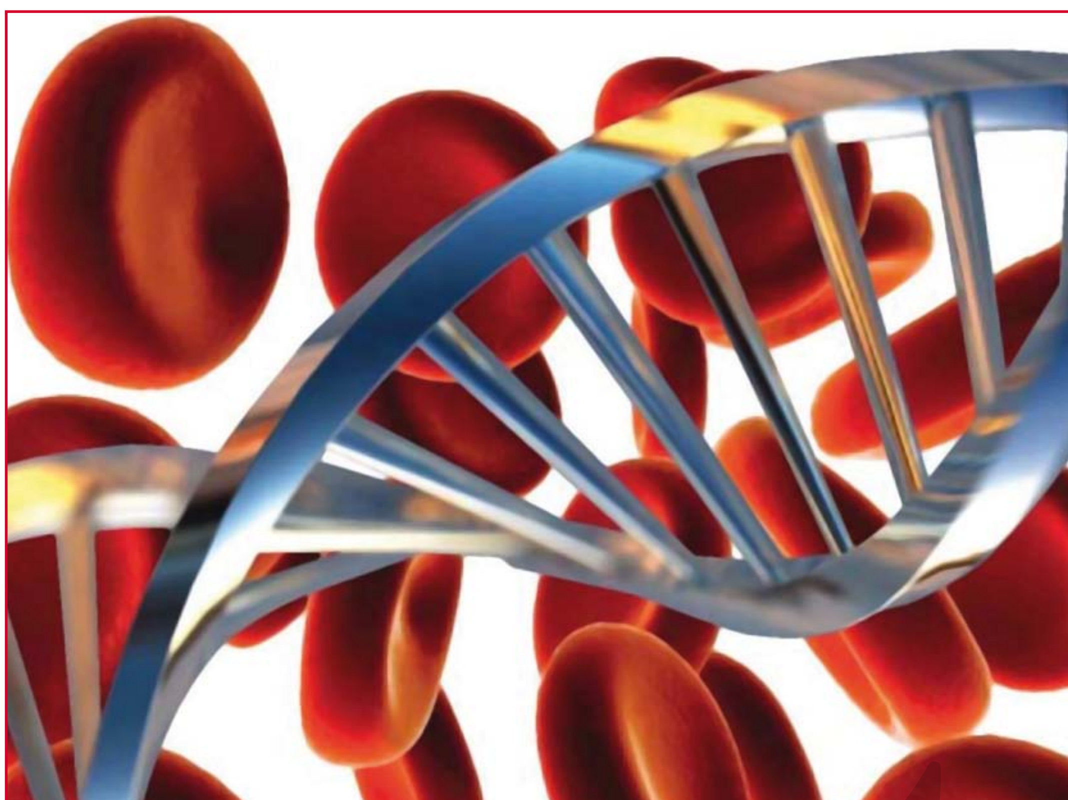
BACKGROUND: Human platelet antigen (HPA) genotyping is important for epidemiological studies because the prevalence of particular HPA allotypes play a major role in the occurrence of HPA alloimmunization which differs among various populations. In Caucasians, antibodies to HPA-1a are the most important cause of neonatal alloimmune thrombocytopenia (NATP). Other studies suggest that anti-HPA-15a/15b and HPA-5b are the most likely candidate antibodies, following anti-HPA-1a, in inducing NATP. To ensure effective transfusion of platelet concentrates in these cases, the availability of platelet donors typed for HPA antigens is of utmost importance. However, their identification is difficult for scarce availability of typing antisera.

AIMS: In January 2008, the Regional Rare Blood Donor Bank and Registry started mass platelet genotyping of donors in Lombardy with the aim of improving the ability to identify antigen negative donors for transfusion support of NATP cases. We used the phenotypes determined for these purpose to estimate the prevalence of these platelet antigens in the Italian population.

METHODS: We used a high- throughput genotyping platform based on DNA microarray. The HPA system genotype was determined by HPA BeadChip™ kits (BioaArray Solutions Ltd. Warren, NJ, USA) designed and manufactured for typing platelets antigens. DNA samples from 15.333 blood donors were typed for the extended antigenic platelet profile HPA-1, 2, 3, 4, 5, 6, 7, 8, 9,11 and 15.



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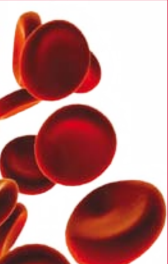
RESULTS: In Table 1 antigenic frequencies for the typed antigens are shown.

HPA GENOTYPE	N.OF DONORS	GENOTYPE FREQUENCY %	HPA ALLELES	ALLELE FREQUENCY %
HPA 1a/a	10.916	71,19%	HPA 1a	84,35%
HPA 1b/b	379	2,47%	HPA1b	15,63%
HPA 1a/b	4.036	26,32%		
HPA 2a/a	12.052	78,60%	HPA 2a	88,61%
HPA 2b/b	200	1,30%	HPA 2b	11,32%
HPA 2a/b	3.070	20,02%		
HPA 3a/a	6.445	42,03%	HPA 3a	64,67%
HPA 3b/b	1.950	12,72%	HPA 3b	35,35%
HPA 3a/b	6.941	45,27%		
HPA 4a/a	15.259	99,52%	HPA 4a	99,54%
HPA 4b/b	0	0,00%	HPA 4b	0,02%
HPA 4a/b	7	0,05%		
HPA 5a/a	11.687	76,22%	HPA 5a	87,33%
HPA 5b/b	238	1,55%	HPA 5b	12,67%
HPA 5a/b	3.408	22,23%		
HPA 6a/a	15.316	99,89%	HPA 6a	99,91%
HPA 6b/b	0	0,00%	HPA 6b	0,02%
HPA 6a/b	5	0,03%		
HPA 7a/a	15.315	99,89%	HPA 7a	99,93%
HPA 7b/b	0	0,00%	HPA 7b	0,05%
HPA 7a/b	14	0,09%		
HPA 8a/a	15.314	99,88%	HPA 8a	99,93%
HPA 8b/b	0	0,00%	HPA 8b	0,05%
HPA 8a/b	16	0,10%		
HPA 9a/a	15.214	99,22%	HPA 9a	99,60%
HPA 9b/b	0	0,00%	HPA 9b	0,38%
HPA 9a/b	114	0,74%		
HPA 11a/a	15.324	99,99%	HPA 11a	99,94%
HPA 11b/b	0	0,00%	HPA 11b	0,01%
HPA 11a/b	1	0,01%		
HPA 15a/a	3.712	24,21%	HPA 12a	48,79%
HPA 15b/b	4.075	26,58%	HPA 12b	51,16%
HPA 15a/b	7.539	49,17%		

CONCLUSIONS: The antigenic frequencies of Italian blood donors are similar to the Caucasian population of other European countries. HPA genotyping provides a good example of molecular based assays that can provide an immediate clinical diagnosis for NATP treatment.



A MULTI-CENTRE STUDY COMPARING THE PERFORMANCE OF THE BIOARRAY HEA BEADCHIP™ ASSAY WITH ESTABLISHED METHODS FOR DETERMINATION OF RED BLOOD CELL ANTIGENS

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BACKGROUND: The BioArray HEA BeadChip assay is a multiplex test that predicts the phenotypes of 38 red blood cell antigens and variants by detecting mutations in DNA. This assay has been available in the USA and Brazil for research use only (RUO), but until now, little data on its performance with the European population has been obtained.

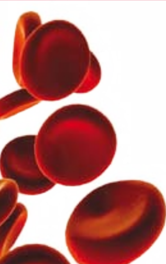
AIMS: This study was performed to determine the performance of this assay for predicting phenotypes in a European population as required by the In Vitro Diagnostic Medical Devices Directive 98/79/EC (IVDD). As far as possible, the BeadChip results were compared to those obtained with CE marked devices.

METHOD: Comparison testing was performed at three institutions, Ospedale Maggiore Policlinico, Milan, Italy , Institut National de la Transfusion Sanguine/Centre National de Référence sur les Groupes Sanguins, Paris, France and Centro de Transfusión Cruz Roja Española, Madrid, Spain. The paired results were obtained by testing samples in parallel with the HEA BeadChip test and the reference method. For the C, c, E, e and K antigens, the number of tests performed and mixture of sample types (patient, donor, neonate) was determined according to the specification in the Common Technical Specifications (2009/108/EC) associated with the IVDD. The reference methods included CE marked serological tests including automated Galileo (Immucor), Autovue Innova (Ortho) and WADiana (Grifols) and manual and DNA-based tests including PCR-SSP assays (Inno-Train, BAG) and ASP and real-time PCR tests.
Results: A total of 1100 samples were tested, 135 of which were patients (31 being neonates) and the remainder were donors. The numbers of compared results differ between antigens because complete comparative data were not available for all antigens and variants in every sample included in the scope of the BeadChip kit. Valid comparison data were available for 24 antigens C, c, E, e, K, k, Kpa, Kpb, Jsa, Jsb, Fya, Fyb, Jka, Jkb, M, N, S, s, Lua, Lub, Doa, Dob, Coa and Cob). These were used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for determination of each antigen.
Overall, 12117 of 12138 antigen determinations (99.82%) were fully congruent between BeadChip and comparative methods. 16 of 21 discrepancies occurred in the Lutheran system, where serological reactions were negative, while the BeadChip and SSP-PCR methods predicted the samples to be antigen-positive.

CONCLUSION: The results of this comparison study showed that the HEA BeadChip HEA assay generates results consistent with established reference methods. Using these performance data, together with additional verifications for the antigens where valid comparison data were not available during this study, BioArray Solutions was successful in attaining the approval from an IVD notified body for CE marking of the HEA BeadChip test as an in-vitro diagnostic medical device including Annex II List A (C, c, E, e, K) and List B (Fya, Fyb, Jka, Jkb) antigen determinations.

TABLE 1 - Parameters of diagnostic efficacy calculated from the comparative data.

ANTIGEN		POSITIVES	NEGATIVES	SENSITIVITY	SPECIFICITY	PPV	NPV
C	RH2	551	477	100%	100%	100%	100%
c	RH4	660	368	100%	99.7%	99.9%	100%
E	RH3	288	735	100%	100%	100%	100%
e	RH5	975	48	100%	100%	100%	100%
K	KEL1	106	766	100%	100%	100%	100%
k	KEL2	622	70	100%	98.6%	99.8%	100%
Kpa	KEL3	29	341	100%	100%	100%	100%
Kpb	KEL4	562	0	100%	N.A.	N.A.	100%
Jsa	KEL6	2	357	100%	100%	100%	100%
Jsb	KEL7	555	2	100%	100%	100%	100%
Fya	FY1	445	405	100%	99.8%	99.8%	100%
Fyb	FY2	665	185	99.7%	100%	100%	99.0%
Jka	JK1	601	252	100%	100%	100%	100%
Jkb	JK2	587	266	100%	100%	100%	100%
M	MNS1	467	141	100%	100%	100%	100%
N	MNS2	419	189	100%	100%	100%	100%
S	MNS3	419	393	100%	100%	100%	100%
s	MNS4	692	120	100%	100%	100%	100%
Lua	LU1	59	398	100%	99.5%	96.7%	100%
Lub	LU2	562	67	100%	82.7%	97.6%	100%
Dia	DI1	0	2	N.A.	N.A.	N.A.	N.A.
Dib	DI2	2	0	N.A,	N.A.	N.A.	N.A.
Coa	CO1	526	22	100%	95.7%	99.8%	100%
Cob	CO2	52	352	100%	100%	100%	100%
Doa	DO1	229	129	100%	100%	100%	100%
Dob	DO2	289	68	100%	100%	100%	100%
Hy	DO4	0	0	N.A.	N.A.	N.A.	N.A.
Joa	DO5	0	0	N.A.	N.A.	N.A.	N.A.
LWa	LW5	0	0	N.A.	N.A	N.A.	N.A.
LWb	LW7	0	1	N.A.	N.A	N.A.	N.A.
Sc1	SC1	1	0	N.A.	N.A	N.A.	N.A.
Sc2	SC2	2	353	100%	100%	100%	100%.



MULTI-PARAMETRIC STUDY FOR VALIDATION OF BIOARRAY HEA BEADCHIP DNA BASED METHOD FOR DETERMINING BLOOD GROUP PHENOTYPES

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[1]Immucor, Paris,France ; [2]Cnrgs, Paris, France

BACKGROUND: The Centre National de Référence pour les Groupes Sanguins (CNRGS) evaluated the BioArray HEA (Human Erythrocyte Antigen) BeadChip ™ (Immucor). The BioArray HEA BeadChip test is a qualitative PCR array based method for determining blood group phenotypes: the results obtained are interpreted depending on the presence or absence of fluorescent signals This laboratory works in compliance with International Standard ISO 17025. In such a laboratory environment, all new methods, before being applied at a routine level must be assessed and validated. Any validation requires the determination of performance criteria and a risk assessment (Fishbone Diagram 6M: Machine, Method, Material, Measurement, Mother nature and Manpower). The outcomes allow determining the critical points needing to be evaluated.

AIM: To validate the BioArray HEA BeadChip tests as a routine method using a multi-parametric study after critical point determination.

METHOD: From the risk assessment, the 3 following critical points were identified:
– Quantity of DNA analyzed
– Annealing temperature in the thermal cycler for the multiplex-PCR step
– Hybridization and elongation temperature in the hybridization oven
The instructions for use state that DNA concentration should be within the range 10-80 ng/µL; the annealing temperature in the thermal cycler has to be 60ºC and the hybridization/elongation step in the oven needs to be 53ºC.

The multi-parametric study included 2 different DNA concentrations, 10 and 80 ng/µL, the annealing was tested at 59,5ºC, 60,0ºC and 60,5ºC. Finally, 52,0ºC, 53,0ºC and 54,0ºC were assessed for the hybridization/elongation step. Three different samples were chosen for their known antigen combinations (see Table 1). Negative controls (H20) were included for technical validation purpose.

RESULTS: For all tests, the negative controls were valid (no amplification was reported), so the study results were valid and can be interpreted.
The data showed the method produced the expected results over the permitted range for each of the parameters studied, and for all combinations of variation within permitted values:
– From 10 to 80 ng/µL of DNA in the sample
– From 59,5 to 60,5ºC during the annealing step
– From 52,0 to 54,0ºC during the hybridization/elongation

CONCLUSION: Results of this study showed the BioArray HEA BeadChip assay to be robust, as critical parameters can be varied to the limits of their permitted ranges without affecting the predictive phenotypes obtained from the genotypes reported. Validation was successfully completed and the assay has been implemented as a routine process. This study can help any laboratory working under international standards to validate and implement the BioArray HEA BeadChip.

SAMPLES	RH1	RH2	RH3	RH4	RH5	KEL1	KEL2	KEL3	KEL4	FY1	FY2	JK1	JK2	MNS1	MNS2	MNS3	MNS4
SAMP 1	+	+	+	+	+	+	+	-	+	+	+	W	+	+	+	-	+
SAMP 2	+	+	-	+	+	-	+	-	+	-	-	+	+	+	+	-	+
SAMP 3	-	-	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+

W: weak

HUMAN ERYTHROCYTE ANTIGEN (HEA) DETERMINATION OF 152 REAGENT RBC SAMPLES WITH HEA BEADCHIP™ DNA ANALYSIS

Ermelina Enriquez, Tama Copeland, Ghazala Ishtiaq, Tasmia Shariff, Ruth Huang, Jarred Lyons, Tyler Hutchinson and Jiacheng Yang

BioArray Solutions, an Immucor Company, Warren, NJ, USA. Red Cell Laboratories at Immucor, Norcross, GA, USA.

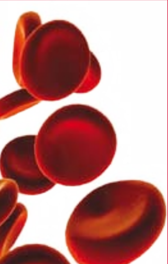
BACKGROUND: DNA analysis permits the simultaneous determination of alleles of genes encoding human red cell antigens. This analysis has been shown to permit the expeditious analysis of large numbers of donors as well as recipients and the reliable prediction of extended human erythrocyte antigen (HEA) phenotypes.

AIMS: The aim of this study was to compare extended serological phenotypes and HEA BeadChip determination for an extended set of antigens detected on the HEA BeadChip panel (C, c, E, e, K, k, Kpa, Kpb, Jsa, Jsb, Fya, Fyb, Jka, Jkb, M, N, S, s, Lua, Lub, Dia, Dib, Coa, Cob, Doa, Dob, Joa, Hy, LWa, LWb, SC1, SC2, and one marker for hemoglobin S).

METHODS: Samples (n=152, obtained from the Red Cell Laboratories at Immucor and several purchased sample panels) polymorphic for human erythrocyte antigens were tested in triplicate, over varying DNA concentrations, following the procedures described in the HEA BeadChip assay package insert. Results for each sample were then tabulated and compared to the reference typing supplied.

RESULTS: Of the reference typing supplied for each antigen and sample, assay sensitivity ranges from 98.72% to 100.00% (mean=99.91%) and assay specificity ranges from 93.08% to 100.00% (mean=99.36%) were obtained. All assay results that met the assay criteria as stated in the product's package insert were used. An initial concordance rate of 99.98% (of n=24,191 reportable results) yielded a final concordance rate of 100% upon resolution of discordant results.

SUMMARY: The HEA eMAP BeadChip assay has been demonstrated to be a robust and reliable method for obtaining predicted phenotypes. The assay sensitivity and specificity demonstrated by this study, coupled with the assay's ability to detect thirty-eight Human Erythrocyte Antigens and phenotypic variants, make the HEA BeadChip a powerful tool in transfusion medicine.



Abst

ERYTROCYTE AND PLATELET GENOTYPING: THEIR UTILITY IN A BLOOD DONOR CENTER AND TRANSFUSION SERVICE

Polo, Ana, Ruiz Ayala, M.L. , Severo, I , Lanes, V , Ruiz Ibafiez, M.E. , Morales, G , Femàndez Ortega

Centro de Translusiòn Banco de Sangre de La Rioja

BACKGROUND: The “Centro de Transfusión Banco de Sangre de La Rioja” acts as Blood Donor Center and Transfusion Service. We process all samples from blood donors and patients (pregnant women, infants, patients with transfusion requirements, etc...) at a routine level. We considered it necessary to implement a molecular biology technique for detection of red cell and platelet antigens, and we validated the BioArray HEA and HPA BeadChipTM techniques (Immucor) in our laboratory.
First, there was a period of staff training, with the assistance of an experienced molecular specialist from Immucor. The training lasted one week and included theoretical and practical sessions during which we used real samples from donors. No incident emerged during the training. In the next following 10 days, the blood bank started routine without any technical supervision from Immucor.

MATERIAL AND METHODS: The BeadChip technique allows performing erythrocyte and/or platelet genotyping.
Erythrocyte genotyping was performed on 140 whole blood samples from donors and patients. Platelet genotyping was done on 14 platelet samples from aphaeresis donors.
Each batch requires the introduction of process controls, one positive and one negative.
The time needed to carry out the complete process has been evaluated; leaving aside the time to select the samples, the labeling and traceability tasks because we feel that this time is common in all genotyping techniques.
We also considered the benefits of the method for the samples analyzed and the difficulties in resolving questions during the process.

RESULTS: Here are the processing times we evaluated:
- The DNA extraction for 24 samples required approximately 3 hours
- The BeadChip process (labeling, multiplex-PCR, post-PCR steps, elongation, reading and interpretation) for 46 samples (with 1 negative and 1 positive controls) required about 1 working day (7 hours)*
* the time needed to complete the process is lower than 5 hours but because of the BeadChip method flexibility we were able to organize the batches to suit the laboratory’s workload

The tables reflect the number of processed samples.

Erythrocyte genotyping:

Nr. of Samples	*Positive Controls*	*Negative Controls*	*Valid Results*
120	*4*	*4*	*120*

Platelet genotyping:

Nr. of Samples	*Positive Controls*	*Negative Controls*	*Valid Results*
120	*4*	*4*	*120*

During the process, we didn’t notice any difficulty neither regarding the sample handling nor with the result interpretation.

CONCLUSIONS:
1. HEA and HPA BeadChip™ are fast and useful to solve certain cases: transfusion incompatibility because of auto-antibodies, chronically transfused patients, doubtful phenotypes...
2. The choice of genotyping to determine erythrocyte or platelet antigens speeds up the work and fulfills the requirements of all particular cases.
3. The genotype and predictive phenotype are released for each test.
4. In our blood donor center, the RBC genotyping would allow us to eliminate the current phenotyping which must be done twice for each donor in order to release the final results.
5. For our needs we considered that the performances are fine.
6. We are currently working on the RHD BeadChip validation

DO EXTENDED-MATCHED RED BLOOD CELLS (RBC) IMPROVE TRANSFUSION YIELD?

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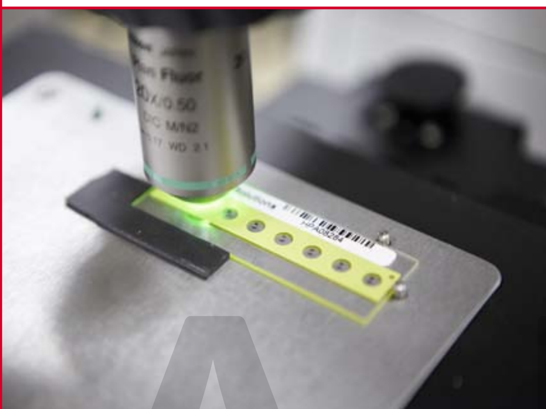
ASP 7 Ragusa, Italy

BACKGROUND: 193 patients with hemoglobinopathies are supported in our Institute, 76 of these have Thalassemia Major (TM). Treatment of this condition is based on a transfusion regimen which guarantees a minimum pre-transfusion hemoglobin (Hb) level of 9g/dL. On average, a dose of 9.8mL 100% concentrated RBC/Kg body mass (range 7.6-12.5) is used, a transfusion interval of 24.2 days (range 18-28) is obtained, and units are matched for AB0, DCE and Kell groups. According to Castilho et al, (ISBT Congress, Berlin 2010), an extended match including additional antigen systems like Duffy, Kidd, MNSs, Dombrock and Lutheran would be expected to improve the transfusion yield in such patients.

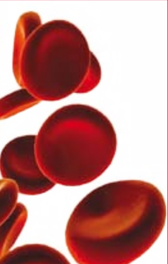
AIMS: Since the transfusion interval can significantly differ between patients, even when subjected to the same transfusion therapy, our first goal is to determine if extended blood matching improves the transfusion yield measured as daily decrease of Hb, with consequent lengthening of transfusion interval and maintenance of clinical pre-transfusion parameters. An additional aim of this study is to determine the number of these patients having a “perfect match” in the donor pool, who could benefit from transfusion therapy with higher compatibility. Since each patient has to be treated with several units/year, this number can be limited by the need for multiple donors matched to each recipient

METHODS: Starting in July 2010, we have adopted a large scale DNA-based method for erythrocyte antigen typing (BioArray HEA BeadChip ™ - Immucor), which is able to determine 38 antigens and variants typically expressed on RBCs.



RESULTS: We have typed 30 TM patients and about a thousand repeat donors as part of a larger project aimed to type approximately 2,000 donors per year. Electronic match between patients and donors has helped to identify 6 individuals showing extended compatibility with at least 40 donors, who can be candidates to sustain extended-match transfusion therapy in these subjects. Four TM patients have been further selected who share similar body weight (50Kg), pre-transfusion Hb levels (9.0-9.5gr/dL) and RBC dosage (550-560mL, 11mL/Kg), show no allo-antibodies and at the same time are not heart-diseased or splenectomized. Two patients (group 1) show a transfusion interval of 28 days (13 transfusions/year, 7150mL packed RBC/year, calculated Hb decrease 0.13gr/day), while the remaining two (group 2) show a transfusion interval of 21 days (17.4 transfusions/year, 9570mL packed RBC/year, calculated Hb decrease 0.17gr/day).

SUMMARY: Since clinical conditions are comparable in the 4 selected patients, the higher need of blood units in group 2 could be a consequence of mismatched minor antigens in previous transfusions. DNA-based extended typing which easily reveals presence or absence of many antigens, including some that cannot be typed serologically, allows the extent of donor-patient mismatch to be determined exactly. Future steps of the present work will be: increase the pool of extensively typed donors to 3000 individuals; treat the 4 selected TM patients for at least one year with extended-match RBCs; compare transfusion yield and intervals (a) with historical data in each single patient, (b) between patients of group 1 and 2.



FREQUENCY OF PLATELET ANTIBODIES IN IMMUNE DISEASES

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BACKGROUND: Screening of platelet (PLT) antibodies was implemented in our department in December of 1997. A total of 1203 samples were processed until December 2010.

AIMS: Present the frequency of positive results in patients with immune diseases concerning a period of 7 years (January 2004 - December 2010). Comment the presence of bleeding manifestations, immunomodulatory therapy and splenectomy. Categorize patients by diagnoses, gender, age and PLT count ranges.

METHODS: Solid phase red cell adherence (SPRCA) – Immucor Inc., instrument-reagent system - was applied for the screening of PLT antibodies. This survey only considers patients of our Hospital and situations where both techniques (Tech) - direct (Dir) and indirect (Ind) - were carried out. Haemograms were determined in ABX Pentra 80/60 and Coulter® LH 750. Samples obtained in ethylenediamine tetraacetic acid.

RESULTS: In the period concerning this survey we assayed a total of 455 samples. In 55 samples it was only possible to perform the Ind Tech due to an insufficient volume of blood or a very low PLT count and in 2 samples it was only performed the Dir Tech due to technical problems. Out of the 455 samples, 63 samples were positive by Ind Tech, 29 samples by Dir Tech and 5 samples by both Tech. From the 455 samples evaluated, we assessed 96 samples corresponding to 72 patients with immune diseases. In this group, diagnoses were expressed per samples/patients as follows: immune thrombocytopenic purpura (ITP) - 44/35, systemic lupus erythematosus (SLE) - 9/7, antiphospholipid syndrome (APS) - 1/1, Sjögren syndrome (SS) - 1/1, ITP+SLE+APS - 8/1, ITP+SS/Multiple Myeloma/APS/kidney transplant - 7/4, ITP+autoimmune haemolytic anaemia - 5/5, SLE+APS/SS/human immunodeficiency virus infection - 6/5, vasculitis - 5/5, vasculitis+kidney cancer - 2/1, rheumatoid arthritis - 1/1, others - 7/6. Considering the evaluated patients, bleeding manifestations occurred in 18, immunomodulatory therapy as corticosteroids, intravenous immunoglobulin, rituximab or other drugs in 32, splenectomy in 5 and blood transfusion in 9. Gender distribution: 52 females and 20 males. Age range: 16-85 years. PLT count: 6-284×109/L. Positive results: 41.7% in samples and 48.6% in patients. We achieved 57.1% in ITP and 40% in ITP with the previously referred associatons; we found 28.6% in SLE and 50% in SLE with the previously referred associations. One patient has ITP+SLE+APS which correspond to an inclusion in both sets analysed. Some information available in the table.

CONCLUSIONS: Concerning the diversity of methods, recent surveys reported frequencies of PLT antibodies in thrombocytopenic patients of 65% in ITP and 69% in SLE. When patients with or without thrombocytopenia were evaluated, the frequencies were lower (<50%). Some authors detected 50% of positivity by SPRCA in ITP. Our results consider patients independently of PLT count and are similar to other studies. The method applied is easy, quick, has a good sensitivity and is useful for screening. When the purpose is to identify the specificity of detected antibodies, it will be necessary to perform other methods. Note: in the text, "/" corresponds to "or" when we present the diagnoses.

SPRCA - Resulls (Samples, Patients)

PL T Antibodies - Positive Results in Immune Diseases		
	Samples	*Patients*
Dir Tech	*9*	*9*
Ind Tech	*23*	*21*
80th Tech	*4*	*3*
Ind Tech (1 st sample) and Dir Tech (2nd sample)	*4*	*2*
Total	*40*	*35*

METHOD FOR QUICK IDENTIFICATION OF ANTI-HPA-1A ALLO-ANTIBODIES : AUTOMATED CAPTURE P RS® ASSAY ON THE GALILEO ECHO.

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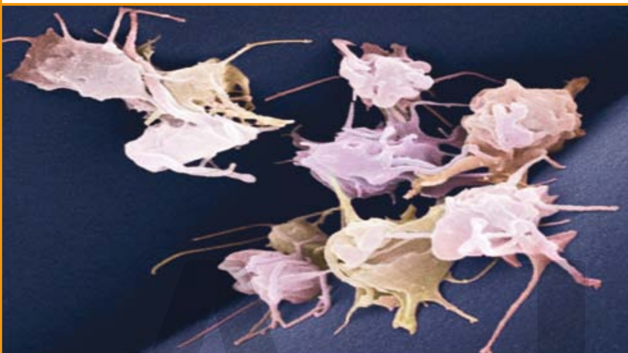
BACKGROUND : HPA (Human Platelet Antigens) are involved in immune conflict such as fetal/neonatal alloimmune thrombocytopenia (FNAIT). Maternal antibodies against HPA-1a causing FNAIT are the most frequent and cause the most morbidity. So, rapid and accurate diagnosis is needed to determine appropriate treatment. Serological investigations are usually performed with a sensitive technique such as the antigen capture enzyme linked immunosorbent assay, namely, the monoclonal antibody immunobilization of platelet antigen's technique (MAIPA), that is considered to be the gold standard assay. However, this technique is manual, relatively slow and variable from technician to technician. The Capture P RS® assay(C-PRS) is a new qualitative immunoassay to detect IgG anti-HLA and anti-HPA allo-antibodies. The kit contains a panel of 13 platelets with known HLA and HPA specificities. It can be associated to Galileo Echo instrument, a fully automated bench top, continuous access platform. Immucor's reagent; HLA Assassin® have been proposed to strip HLA class I from platelets, revealing specifically anti-HPA allo-antibodies.

AIM : to evaluate the identification of anti-HPA-1a allo-antibodies by using the Automated Capture P RS® assay on the Galileo Echo.

METHODS : 50 sera, including 10 that have no allo-antibodies anti-HLA class I and anti-HPA, 10 with allo-antibodies anti-HLA class I detected by Cytotoxicity dependent-Complement and/or by Luminex (LifeCodesLSATM HLA class I, Gen-Probe) and 15 with allo-antibodies anti-HPA-1a and 5 with other anti-HPA and 15 auto-antibodies anti-platelet glycoproteins detected by MAIPA were studied by Automated Capture P RS Assay on the Galileo Echo.

RESULTS : All samples without allo-antibodies were not reactive before and after HLA assassin reagent incubation in C-PRS assay. Samples with anti-HLA antibodies detected by CDC and not by Luminex demonstrated positive tests in C-PRS Assay. After Incubation of HLA assassin reagent, only 1/10 sample with anti-HLA-A28 remained positive, demonstrating the efficacy of removal of HLA antigens. Auto-antibodies anti-platelet glycoproteins were not detected by C-PRS, so did not interfere with the detection of anti-HPA allo-antibodies. All anti-HPA-1a allo-antibodies (15/15 samples) were correctly identified after HLA assassin reagent incubation. 5/15 samples before HLA assassin reagent incubation were reactive with other (HPA-1a negative) platelets because of the presence of anti-HLA antibodies. Titration studies showed that the sensitivity of C-PRS for detecting Anti-HPA-1a was two dilutions superior to that of MAIPA.

CONCLUSION : This study confirms that the HLA assassin reagent allows the removal of HLA specific antigens, without the destruction of platelet antigenic determinants. The C-PRS is applicable for acute diagnosis of FNAIT using the HLA assassin reagent, especially to detect anti-HPA-1a allo-antibodies. The technique is fast (30 mn) and very easy. The interpretation is very user-friendly.



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COMPARISON OF THE INTENSITY OF ANTI-RH1 DETECTION USING GALILEO (IMMUCOR) AND GEL MICROTITRATION: A SEMI QUANTITATIVE AUTOMATED APPROACH TO DETERMINE THE ORIGIN OF ANTI-RH1.

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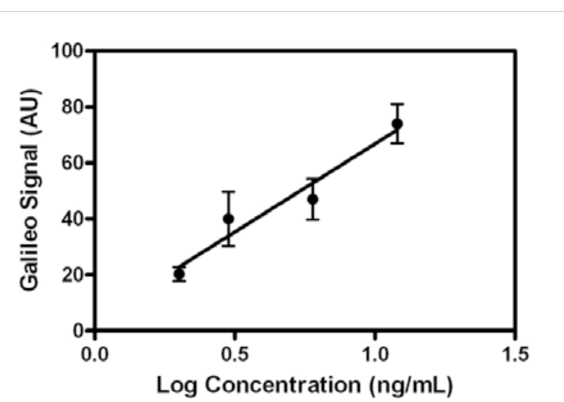
BACKGROUND: In 2005, the French National College of Gynecologists and Obstetricians published guidelines recommending systematic injections of anti-D immunoglobulins at 28 weeks of pregnancy for all RH:-1 women. Since, the rate of positive antibody screening has increased. It therefore becomes essential to differentiate passive anti-RH1 from allo-anti RH1. In order to resolve this issue the CNRHP developed in 2000, the anti-RH1 microtitration method, a semi-quantitative assay used to determine if an anti-RH1 results from administration of IgRH or from alloimmunization.
Aim: The intensity of agglutination of antibody screening using Capture® technology on Galileo® (Immucor) was compared to a microtitration method in pregnant women serum (injected?) with anti-RH1.

METHODS: Blood samples of pregnant women were screened for antibodiesusing Capture-R (4 cell) technology on Galileo. Samples with anti-RH1 were then analysed by microtitration using gel column technology. For each range of concentration, the mean of the Capture-R agglutination intensities was calculated and then compared to the result of the microtitration.

RESULTS: 37 anti-RH1 positive samples were analysed with both methodologies: 6 samples at 1.5 ng/ml, 9 at 3 ng/ml, 15 at 6 ng/ml, 7 at 12 ng/ml. A good correlation was found between the 2 methods $R^2 = 0.948$ with the logarithm function: Slope 63.00 ± 10.83, Y-intercept 3.815 ± 7.827, X-intercept -0,06055, 1/slope 0,01587



CONCLUSION: The work presented here is a first step before a complete validation of the method. These preliminary results indicate that the fully automated Galileo technology can be used to design a semi quantitative approach of anti RH1 determination. The concentration found by the Galileo can be compared with the expected concentration of anti RH1 following IgRHD injection knowing both the date and the dose of the injection. If the result of the Galileo measurement exceeds the expected concentration, an alloimmunization can be suspected. This method will be very useful in the monitoring of RH:-1 women when IgRH have been previously injected.

DETECTION OF DEL PHENOTYPES BY AN AUTOMATED SOLID PHASE ADHERENCE METHOD

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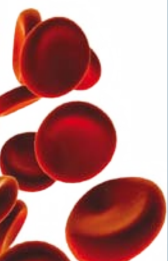
BACKGROUND: Rh is the most important blood group system after ABO in transfusion medicine. The polymorphism of the Rh blood group system is well known and is deeply and continuously studied with serological and molecular techniques. Among the D antigen variants, one with the smallest numbers of D antigen sites expressed on the red blood cell membrane, is called DEL, which is found in 10%-33% of Japanese and Chinese RhD negative by conventional serological techniques. This variant derives from different RHD mutations and can only be detected by very sensitive serological techniques, in particular absorption and elution, or by DNA typing.
Aim: Eight samples were initially referred to us because they showed unexpected serological positivity in the Weak D indirect antiglobulin test (Weak D IAT) performed with Solid Phase Red Cell Adherence (SPRCA) with a monoclonal IgG anti-D, clone D415 (ImmucorGamma, Norcross, GA, USA), but were negative with other methods. We reproduced these results and found by DNA analysis that these carried the DEL(M295I) mutation. We investigated these samples in parallel with 5 known DEL samples from our own facility.

METHODS: We tested the 13 samples serologically at room temperature (RT), using a microplate technique (Galileo, ImmucorGamma) with anti-D clones D175 and MS 201 and in CAT BioVue [Innova, Ortho Clinical Diagnostics (OCD), Raritan, NJ, USA] with anti-D clones TH28 and RUM1. We performed Weak D IAT in SPRCA (Capture R Select, ImmucorGamma) with anti-D clones MS26 and D415 and CAT BioVue with anti-D clones LDM1 and LDM3, OCD. We confirmed the phenotype with absorption/elution: 1 hour incubation at 37 °C with anti-D Novaclone (ImmucorGamma) without additive; absorption performed with CcDee red blood cells (RBCs); eluate obtained with Elu-Kit (ImmucorGamma) tested with 4 RhD+ RBCs and 4 RhD- RBCs. We confirmed the genotype with RHType kit (BaGene, BAG Health Care GmbH, Lich, Germany).

RESULTS: All 13 DEL samples showed negative reactions, as expected, with all tests at RT. Positive reactions were observed only using the Weak D IAT performed in SPRCA with anti-D Novaclone (containing IgG clone D415). These samples were all confirmed serologically by both absorption/elution tests and by genotyping as DEL (M295I).

CONCLUSIONS: Weak D IAT performed in SPRCA with anti-D Novaclone (IgG clone D415) has been proven as the only other serological test available to give a positive reaction with DEL (M295I). Some DEL variants could induce an alloanti-D in RhD-recipients because most DEL donors are typed as RhD- by serological method. Therefore, the introduction of easy, automated serological test into routine screening could contribute to prevent diagnostic errors where it is not possible to perfom RhD genotyping.



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WIDESPREAD STUDY ON THE RHD VARIANTS IN ITALY: WWW.PROGETTO-ARCOBALENO.IT

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BACKGROUND: The "Arcobaleno Project" is a widespread study on the RHD antigen variants and their frequency while taking into account immigration and the globalization of health care in Italy. 21 Transfusion Services (TS) collaborate in the project in 8 regions (Lazio, Campania, Tuscany, Piedmont, Lombardy, Friuli, Veneto, Trentino). The centers are identified by a code and upload the serological and molecular typing results into the website www.progetto-arcobaleno.it. Some meetings are organized among the TS for discussion and updates.

METHODS: In accordance with the operating protocol the selected samples from the level I routine RHD typing are performed by microplate or micro-coloumn on an automatic analyzer (Galileo, Echo and Neo-Immucor, Autovue Innova-Ortho, Techno-Diamed, Tango-Biotest). They are then sent for further serologic investigations by anti-D mono and polyclonal sera of different companies: Immucor, Biotest, Diagast, Diamed, Ortho, Formedic. 12 anti-D IgG sera in solid phase (Albaclone Advanced Partial RhD Typing) using Capture Select (Immucor), 6 anti-D sera in gel card (RhD partial Diamed) and molecular (SSP-PCR by BAG and Innotrain kits) advanced investigation were also carried out.

RESULTS: A total of 975 individuals (522 male/453 female) were analyzed: 63% were blood donors and 37% were patients. 808 (85%) were identified as weak-D and 147(15%) as partial-D divided as follows: N. 542 (56%) weak-D type1, N. 88(9%) type2, N.76(8%) type3, N.28(3%) type4.0/4.1, N.21(2%) type4.2, N. 26(3%) type5, N. 20(2%) type11, N.7(1%) type 9, 15, 18, 61; N.72(7%) DVII, N.19(2%) DFR, N.10(1%) DVI type1, N.15(2%) DVI type2,4, N.9(1%) DNB, N.7(1%) DAU, N.15(2%) DIV tipo4, DV, DEL, DHAR, D. 25 samples had results which were discrepant between the serological and the molecular typing; these were sent for sequencing testing. Only five of these have been done so far.

CONCLUSIONS: The distribution of RHD variants in Italy shows a higher rate of weak-D type 1,2,3 in five northern regions. In Piedmont the DVI type2 and DFR are more frequent, while in Tuscany the DNB is more prevalent. In Campania the weak-D type5 and DVI type4 are more common. The DVI type4 is a Spanish variant, the presence of which can be explained by historical Spanish domination in this area. Lazio is the region with the highest rate of DVII variant in Italy. The use of sequencing testing makes it possible to identify in five discrepant samples three variants, weak-D types 9,18 and 61, which are not identified with the SSP-PCR kits currently on the market. The molecular investigation needs to be based on an increased number of alleles. It is important to note that the African immigrant patients have variants such as D , DAU, weak-D 4.2 coming from the three phylogenetic clusters. The Arcobaleno Project aims to conduct a serologic and molecular evaluation to agree on some integrated and shared protocols for the RHD typing. Finally, it is necessary to investigate the anti-D alloimmunization rate of the variants and to be able to identify the African or Asian alleles to provide a better immune transfusion service to the foreign patients.

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