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IDCOREXT

PACKAGE INSERT

Kit Product Number: 1021720000

48 Tests

Store at 2-8°C.

IVD

: Progenika Biopharma, S.A.
Parque Tecnológico de Bizkaia
Ibaizabal bidea, Edificio 504
48160 Derio - Bizkaia - SPAIN

GRIFOLS

CONTENTS

1. GENERAL INFORMATION

1.1. GENERAL DESCRIPTION

The ID CORE XT test uses Polymerase Chain Reaction (PCR) amplification to obtain large amounts of the target sequence encoding Human Erythrocyte Antigens (HEAs). The ID CORE XT test relies on allele-specific probes attached to color-coded microspheres, which hybridize specifically to the labeled PCR products. A subsequent fluorescent labeling step allows detection and quantification of the hybridization signal. ID CORE XT generates a simultaneous multiplex reaction in a single well, avoiding the need to run separate methods in parallel. The software outcome is a polymorphism genotype, a predicted allele genotype and a predicted phenotype, bypassing any subjective interpretation of the results.

1.2. INTENDED USE

ID CORE XT™ (Reagents and Analysis Software) is a qualitative, polymerase chain reaction (PCR) and hybridization-based genotyping test for the simultaneous identification of multiple alleles encoding human erythrocyte antigens (HEAs) in genomic DNA extracted from whole blood specimens collected in EDTA.

This test can be used to genotype the polymorphisms and predict the allele genotypes and antigen phenotypes of the blood group systems listed in Table 1, as an alternative to serology.

Table 1. List of the polymorphisms, alleles and antigens interrogated by ID CORE XT.

Blood Group System	Polymorphism	Allele	Antigen (ISBT)
Rh	*RHCE*:c.122A>G *RHCE*:c.307T>C *RHCE*:c.335+3039ins109 *RHCE*:c.676G>C *RHCE*:c.712A>G *RHCE*:c.733C>G *RHCE*:c.1006G>T *RHD-CE-D* hybrid	*RHCE*ce* *RHCE*cE* *RHCE*Ce* *RHCE*CE* *RHCE*CeCW* *RHCE*ceCW* *RHCE*CECW* *RHCE*ceAR* *RHCE*ce[712G]* *RHCE*ce[733G]* *RHCE*Ce[733G]* *RHCE*Ce[712G,733G]* *RHCE*cE[712G,733G]* *RHCE*ce[733G,1006T]* *RHD*r's-RHCE*ce[733G,1006T]* *RHCE*CeFV* *RHCE*cEFM* *RHCE-D[5, 7]-CE*	C (RH2) E (RH3) c (RH4) e (RH5) CW (RH8) V (RH10) hrS (RH19) VS (RH20) hrB (RH31)
Kell	*KEL*:c.578T>C *KEL*:c.841T>C *KEL*:c.1790C>T	*KEL*K_KPB_JSB* *KEL*k_KPB_JSB* *KEL*k_KPA_JSB* *KEL*k_KPB_JSA*	K (KEL1) k (KEL2) Kpa (KEL3) Kpb (KEL4) Jsa (KEL6) Jsb (KEL7)
Kidd	*SLC14A1*:c.342-1G>A *SLC14A1*:c.838G>A *SLC14A1*:c.871T>C	*JK*A* *JK*B* *JK*B_null(IVS5-1a)* *JK*A_null(IVS5-1a)* *JK*B_null(871C)*	Jka (JK1) Jkb (JK2)

Blood Group System	Polymorphism	Allele	Antigen (ISBT)
Duffy	*FY*:c.1-67T>C *FY*:c.125G>A *FY*:c.265C>T	*FY*A* *FY*B* *FY*A_GATA* *FY*B_GATA* *FY*A[265T]* *FY*B[265T]_FY*X*	Fya (FY1) Fyb (FY2)
MNS		*GYPA*M* *GYPA*N* *GYPB*S* *GYPB*s* *GYPB*S_null(230T)* *GYPB*S_null(IVS5+5t)* *GYP*[140A]* *GYPB*deletion*	M (MNS1) N (MNS2) S (MNS3) s (MNS4) U (MNS5) Mia (MNS7)
Diego	*DI*:*c*.2561T>C	*DI*A* *DI*B*	Dia (DI1) Dib (DI2)
Dombrock		*DO*A* *DO*B* *DO*B_HY* *DO*A_JO*	Doa (DO1) Dob (DO2) Hy (DO4) Joa (DO5)
Colton	*CO*:c.134C>T	*CO*A* *CO*B*	Coa (CO1) Cob (CO2)
Cartwright	*YT*:c.1057C>A	*YT*A* *YT*B*	Yta (YT1) Ytb (YT2)
Lutheran	*LU*:c.230A>G	*LU*A* *LU*B*	Lua (LU1) Lub (LU2)

1.3. PRINCIPLES OF THE TEST

ID CORE XT utilizes Luminex xMAP technology. Genomic DNA extracted from human EDTA anticoagulated whole blood is amplified and biotinylated by multiplex PCR. PCR products are denatured and hybridized to oligonucleotide probes coupled to color-coded beads. Hybridized DNA is labeled with a fluorescent conjugate and the resulting signal is detected with a Luminex 200 system. Raw data are processed with the ID CORE XT ANALYSIS SOFTWARE to obtain polymorphism genotypes, predicted allele genotypes and predicted phenotypes for each blood group and HEA interrogated by the test (see Table 1).

The ID CORE XT ANALYSIS SOFTWARE algorithm converts the polymorphism genotypes into predicted allele genotypes and predicted phenotypes for each blood group and antigen, respectively, based on the literature. In addition, ID CORE XT results include explanatory notes for some results outputs, for instance calling attention to the fact that, although unlikely, another allele genotype may be present (with potential implications in the corresponding phenotype), or expression of an antigen is altered (weak, partial or variable), also based on current literature. For more information see ID CORE XT ANALYSIS SOFTWARE User Manual.

Table 2. Description of the polymorphisms, alleles and antigens tested by ID CORE XT.

Blood Group System	Internal Code	ID CORE XT polymorphism	ISBT allele name or/and Reference #	ID CORE XT allele	ISBT phenotype	ID CORE XT antigen (ISBT)
Rh	PS87	*RHCE*:c.122A>G	*RHCE*02.08.01*	*RHCE*CeCW*	RH:8	CW (RH8)
			Ref.1	*RHCE*ceCW*	N.A.	CW (RH8)
			Ref.2	*RHCE*CECW*	N.A.	CW (RH8), hrS(RH19), hrB (RH31)
	PS88	*RHCE*:c.307T>C	*RHCE*01*	*RHCE*ce*	RH:4	c (RH4)
			*RHCE*03*	*RHCE*cE*		
	PS89	*RHCE*:c.335+3039ins109	*RHCE*02*	*RHCE*Ce*	RH:2	C (RH2)
			*RHCE*04*	*RHCE*CE*		
	PS91	*RHCE*:c.676G>C	*RHCE*01*	*RHCE*ce*	RH:5	e (RH5)
			*RHCE*02*	*RHCE*Ce*		
			*RHCE*03*	*RHCE*cE*	RH:3	E (RH3), hrS (RH19), hrB (RH31)
			*RHCE*04*	*RHCE*CE*		
	PS92 PS93 PS182 PS193	*RHCE*:c.712A>G *RHCE*:c.733C>G *RHCE*:c.1006G>T *RHD-CE-D* hybrid	*RHCE*01.04*	*RHCE*ceAR*	RH:10, RH:-19	V (RH10), hrS (RH19)
			*RHCE*01.05/08/09*	*RHCE*ce[712G]*	RH:-19	hrS (RH19)
			*RHCE*02.02*	*RHCE*CeFV*	N.A.	N.A.
			*RHCE*03.03*	*RHCE*cEFM*	N.A.	hrS (RH19), hrB (RH31)
			*RHCE*01.20.01/02*	*RHCE*ce[733G]*	RH:10, RH:20, RH:-31	V (RH10), VS (RH20), hrB (RH31)
			*RHCE*01.20.03/05*	*RHCE*ce[733G,1006T]*	RH:20, RH:-31	VS (RH20), hrB (RH31)
			*RHCE*02.04*/Ref.3	*RHCE*Ce[712G,733G]*	N.A.	N.A.
			Ref.4	*RHCE*cE[712G,733G]*	N.A.	hrS (RH19), hrB (RH31)
			Ref.5	*RHCE*Ce[733G]*	N.A.	V (RH10),
			*RHCE*01.20.03*	*RHD*r's-RHCE*ce[733G,1006T]*	RH:20, RH:-31	VS (RH20), hrB (RH31)
	PS91 PS92 PS93 PS182	*RHCE*:c.676G>C *RHCE*:c.712A>G *RHCE*:c.733C>G *RHCE*:c.1006G>T	Ref.6	*RHCE-D[5, 7]-CE*	N.A.	C (RH2), E (RH 3), c (RH4), e (RH5), CW (RH8), V (RH10), hrS (RH19), VS (RH20), hrB (RH31)
Kell	PS96	*KEL*:c.578T>C	*KEL*01.01*	*KEL*K_KPB_JSB*	KEL:1	K (KEL1)
			*KEL*02*	*KEL*k_KPB_JSB*	KEL:2	k (KEL2)
	PS97	*KEL*:c.841T>C	*KEL*02.03*	*KEL*k_KPA_JSB*	KEL:3, KEL:-4	Kpa (KEL3), Kpb (KEL4)
	PS100	*KEL*:c.1790C>T	*KEL*02.06*	*KEL*k_KPB_JSA*	KEL:6, KEL:-7	Jsa (KEL6), Jsb (KEL7)

Blood Group System	Internal Code	ID CORE XT polymorphism	ISBT allele name or Reference #	ID CORE XT allele	ISBT phenotype	ID CORE XT antigen (ISBT)
Kidd	PS101	*SLC14A1*:c.342-1G>A	*JK*02N.01*	*JK*B_null(IVS5-1a)*	JK:-3	Jkb (JK2)
			*JK*01N.06*	*JK*A_null(IVS5-1a)*	JK:-3	Jka (JK1)
	PS102	*SLC14A1*:c.838G>A	*JK*01*	*JK*A*	JK:01	Jka (JK1)
			*JK*02*	*JK*B*	JK:02	Jkb (JK2)
	PS103	*SLC14A1*:c.871T>C	*JK*02N.06*	*JK*B_null(871C)*	JK:-3	Jkb (JK2)
Duffy	PS104	*FY*:c.1-67T>C	*FY*01N.01*	*FY*A_GATA*	Fy(a-b-)	Fya (FY1)
			*FY*02N.01*	*FY*B_GATA*	Fy(a-b-)	Fyb (FY2)
	PS105	*FY*:c.125G>A	*FY*01*	*FY*A*	FY:1	Fya (FY1)
			*FY*02*	*FY*B*	FY:2	Fyb (FY2)
	PS106	*FY*:c.265C>T	*FY*01W*	*FY*A[265T]*	Fya+w	Fya (FY1)
			*FY*02W*	*FY*B[265T]_FY*X*	Fyx	Fyb (FY2)
MNS	PS108	*GYPA*:c.[59C>T]	*GYPA*01*	*GYPA*M*	MNS:1	M (MNS1)
			*GYPA*02*	*GYPA*N*	MNS:2	N (MNS2)
	PS109	*GYPB*:c.143T>C	*GYPB*03*	*GYPB*S*	MNS :3	S (MNS3)
			*GYPB*04*	*GYPB*s*	MNS :4	s (MNS4)
	PS110	*GYPB*:c.230C>T	*GYPB*03N.01*	*GYPB*S_null(230T)*	MNS:–3; MNS:5W	S (MNS3), U (MNS5)
	PS112	*GYPB*:c.270+5G>T	*GYPB*03N.03*	*GYPB*S_null(IVS5+5t)*	MNS:–3; MNS:5W	S (MNS3), U (MNS5)
	PS113	*GYP. Hybrid*	*GYP.501/ GYPA*09*	*GYP*[140A]*	MNS:7	Mia (MNS7)
	PS109 PS110 PS112	*GYPB*:c.143T>C *GYPB*:c.230C>T *GYPB*:c.270+5G>T	*GYPB*01N*	*GYPB*deletion*	MNS:-3,-4,-5	S (MNS3), s (MNS4), U (MNS5), Mia (MNS7)
Diego	PS114	*DI*:c.2561T>C	*DI*01*	*DI*A*	DI:1	Dia (DI1)
			*DI*02*	*DI*B*	DI:2	Dib (DI2)
Dombrock	PS115	*DO*:c.793A>G	*DO*01*	*DO*A*	DO:1	Doa (DO1)
			*DO*02*	*DO*B*	DO:2	Dob (DO2)
	PS133	*DO*:c.323G>T	*DO*02.–04*	*DO*B_HY*	DO:–4	Hy (DO4)
	PS134	*DO*:c.350C>T	*DO*01.–05*	*DO*A_JO*	DO:–5	Joa (DO5)
Colton	PS116	*CO*:c.134C>T	*CO*01.01*	*CO*A*	CO:1	Coa (CO1)
			*CO*02*	*CO*B*	CO:2	Cob (CO2)
Cartwright	PS136	*YT*:c.1057C>A	*YT*01*	*YT*A*	YT:1	Yta (YT1)
			*YT*02*	*YT*B*	YT:2	Ytb (YT2)
Lutheran	PS48	*LU*:c.230A>G	*LU*01*	*LU*A*	LU:1	Lua (LU1)
			*LU*02*	*LU*B*	LU:2	Lub (LU2)

Notes: When Ref. # is indicated, see the section "References" (these allele names are not described in the ISBT database (Ref.17).
N.A.: ISBT phenotypes and/or ID CORE XT antigens (V, VS, hrB, and/or hrS) are not described.

2. REQUIRED COMPONENTS, MATERIALS AND EQUIPMENT

2.1. KIT COMPONENTS

ID CORE XT (1021720000)	Component	Part Number	Number of Tests	Area	Storage
ID CORE XT Reagents (1020320000)	ID CORE XT PCR Master Mix	1020320100	48 tests	Pre-PCR	2-8ºC
	ID CORE XT Beads Master Mix	1020320200	48 tests	Post-PCR	2-8ºC
	SAPE	0000021600	48 tests	Post-PCR	2-8ºC
	SAPE Dilution Buffer	0000021700	48 tests	Post-PCR	2-8ºC
ID CORE XT Software (1021700000)	ID CORE XT ANALYSIS SOFTWARE	1021700300	Not applicable	Not applicable	Not applicable
	ID CORE XT Package Insert	1021700401	Not applicable	Not applicable	Not applicable
	ID CORE XT ANALYSIS SOFTWARE User Manual	1021700501	Not applicable	Not applicable	Not applicable
	ID CORE XT Luminex template	1020300600	Not applicable	Not applicable	Not applicable

Note: Refer to the expiration date printed on the reagent label, and do not use the kit or any kit components past the indicated expiration date.

2.2. EQUIPMENT REQUIRED

Pre PCR Area	
Product Description	**Manufacturer**
Adjustable pipettes (2 µl to 1,000 µl). Pipette accuracy and precision should comply with ISO 8655 Pipette Standards	None specified
Multichannel pipettes (5 µl to 20 µl). Pipette accuracy and precision should comply with ISO 8655 Pipette Standards	None specified
Non-refrigerated micro centrifuge	None specified
Dry heat block or water bath (might be required for genomic DNA extraction)	None specified
Vortex mixer	None specified
Spectrophotometer	None specified

Post PCR Area	
Product Description	**Manufacturer**
Adjustable pipettes (2 µl to 1,000 µl). Pipette accuracy and precision should comply with ISO 8655 Pipette Standards	None specified
Multichannel pipettes (4 µl to 80 µl). Pipette accuracy and precision should comply with ISO 8655 Pipette Standards	None specified
Vortex mixer	None specified
Non-refrigerated plate centrifuge	None specified
Non-refrigerated micro centrifuge	None specified
Veriti Dx 96-Well Thermal Cycler (4452300)	Thermo Fisher Scientific
Compact flow analyzer: Luminex 200	Luminex
Flow analyzer software: Luminex xPONENT (3.1)	Luminex

2.3. MATERIALS REQUIRED BUT NOT PROVIDED

Pre PCR Area	
Product Description	**Manufacturer**
QIAamp DSP DNA Blood Mini Kit (61104)	QIAGEN
QIAsymphony DSP DNA Mini Kit (937236)	QIAGEN
ID CORE CONTROL (1301790001): ID CORE CONTROL 1 and ID CORE CONTROL 2	Progenika Biopharma
HotStarTaq DNA Polymerase, QIAGEN (700410)	QIAGEN
Nuclease-free 1.5 ml tubes	None specified
96-well reaction plates. For example: MicroAmp Optical 96 well reaction plates. Thermo Fisher Scientific (N8010560)	None specified
Adhesive film. For example: MicroAmp Clear Adhesive film. Thermo Fisher Scientific (4306311)	None specified
Disposable aerosol filter pipette tips	None specified
Nuclease free molecular-grade water	None specified
Multichannel pipette reagent reservoir	None specified

Post PCR Area	
Product Description	**Manufacturer**
Compression pads. For example: MicroAmp Optical compression pad. Life Technologies (4312639)	None specified
Multiplate Low-Profile 96-Well Unskirted PCR Plates (Bio-Rad MLL-9601), low profile 96-well polypropylene clear	Bio-Rad
Microseal “A” Film (MSA-5001)	Bio-Rad
Nuclease-free 1.5 ml tubes	None specified
Multichannel pipette reagent reservoir	None specified
Disposable aerosol filter pipette tips	None specified
15 ml tubes	None specified
xMAP Sheath Fluid (40-5000)	Luminex
Luminex Calibrators and Controls (LX200-CAL-K25 and LX200-CON-K25)	Luminex

3. WARNINGS AND CAUTIONS

- Only personnel qualified as proficient in the use of ID CORE XT assay should perform this procedure.
- The procedure should be performed as described this package insert using the required reagents and the corresponding volumes detailed. Any deviation from the outlined protocols and/or reagents may result in assay failure, and/or cause erroneous results. Accurate pipetting of samples and reagents is required for accurate results. Viscous liquids should be pipetted slowly and gently.
- Perform equipment maintenance following the manufacturer’s recommendations.
- Samples should be treated as potentially infectious. Universal precautions should be observed at all times.
- It is strongly recommended to have two separate work areas: pre-PCR and post- PCR. The workflow must be unidirectional, starting in the pre-PCR area and moving toward the post-PCR area. Equipment and reagents must be exclusive to each area and interchanging them should not be permitted.
- Use of nuclease-free filter pipette tips is recommended to avoid nuclease contamination of reagents and samples in pre-PCR and post-PCR areas.
- Use a refrigerator capable of maintaining a temperature of 2-8ºC to store the kit reagents.
- Use a defrost-free freezer capable of maintaining a temperature of -15ºC to -25ºC or colder to store the DNA samples, Hot Star Taq DNA polymerase and/or the PCR products.
- Ensure HotStarTaq DNA Polymerase part-number is the one required.

- Do not mix or interchange kit components from different kit lots. Do not use the kit or any kit components past the indicated expiration date.
- Keep CDs when out of their cases away from dust, water and direct sunlight, and avoid scratching CDs.
- Discard reagents that have been in use for longer than 6 months.
- Ensure that the Veriti Dx Thermal Cycler has been preprogrammed with ID XT PCR amplification and ID XT HYB hybridization programs (see sections 5.3 and 5.4 for more details). If this was not the case, contact Grifols Technical Service.
- Ensure the PCR plate/hybridization plate/labeling plate is sealed tightly and placed with the PCR compression pads on the thermal cycler block.
  Safety Data Sheets (SDS) are available from Grifols Technical Service upon request. Following the OSHA Hazard Communication Standard (29 CFR 1910.1200), the Regulation (EC) No 1272/2008 [CLP/GHS], and HPR SOR/2015-17, the ID CORE XT Beads Master Mix is classified as follows:

Pictogram	
Signal word	*Danger*

Hazard statements		
H300	Acute toxicity (oral) cat. 2.	*Fatal if swallowed.*
H311	Acute toxicity (dermal) cat. 3.	*Toxic in contact with skin.*
H315	Skin irritation cat. 2.	*Causes skin irritation.*
H319	Serious eye damage/eye irritation cat. 2.	*Causes serious eye irritation.*
H335	Specific target organ toxicity-single exposure cat. 3. Respiratory tract irritation.	*May cause respiratory irritation.*
H370	Specific target organ toxicity- Single exposure cat. 1. (Central Nervous System, oral).	*Causes damage to organs if swallowed.*
H411	Hazardous to the aquatic environment- chronic hazard cat. 2.	*Toxic to aquatic life with long lasting effects.*

Precautionary statements	
P260	*Do not breathe vapors.*
P270	*Do not eat, drink or smoke when using this product.*
P271	*Use only outdoors or in a well-ventilated area.*
P273	*Avoid release to the environment.*
P280	*Wear protective gloves/ protective clothing/ eye protection/ face protection.*
P301+P310+P330	*IF SWALLOWED: Rinse mouth and immediately call a POISON CENTER or doctor/ physician.*
P302+P313+P332+P352	*IF ON SKIN: Wash with plenty of soap and water. If skin irritation persists: Get medical advice/ attention.*
P304+P312+P340	*IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or doctor/ physician if you feel unwell.*
P305+P313+P337+P338+P351	*IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation occurs: Get medical advice/ attention.*
P362	*Take off contaminated clothing and wash before reuse.*
P391	*Collect spillage.*
P403+P405+P233	*Store locked up in a well-ventilated place. Keep container tightly closed.*
P501	*Dispose of contents/containers in accordance with local/regional/national/international regulations.*

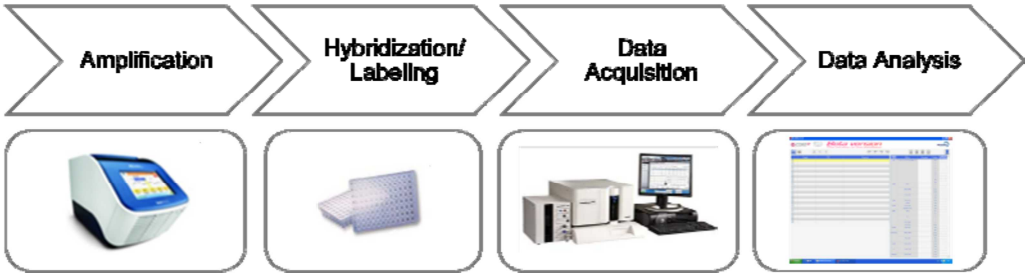
4. STORAGE AND STABILITY

- Store all ID CORE XT reagents at 2-8ºC.
- Refer to the expiration date printed on the reagent label.

5. PROCEDURE

5.1. WORKFLOW

The ID CORE XT protocol consists of 4 steps:



Each batch of samples per reagent lot must be tested with one replicate of each of the two positive control samples included in the ID CORE CONTROL kit (ID CORE CONTROL 1 and ID CORE CONTROL 2), as well as with one replicate of the Negative Control (nuclease-free molecular-grade water) placed at the end of the batch. To avoid incompatibility issues with the ID CORE XT ANALYSIS SOFTWARE, these controls must be exactly named as follows: ID CORE CONTROL 1, ID CORE CONTROL 2, Negative Control.

5.2. SPECIMEN COLLECTION, PREPARATION AND STORAGE

The assay is for use with human genomic DNA extracted from whole blood collected with EDTA as the anticoagulant and stored at 2-8°C.

Genomic DNA extraction should be carried out in the pre-PCR area. Store genomic DNA samples at -15 to -25°C or below.

Use of QIAamp DSP DNA Blood Mini Kit or QIAsymphony DSP DNA Mini Kit, a DNA purity range (A260/A280) of 1.65 to 2.00, and a dilution to 20 ng/µl is recommended for optimal performance. Use of alternative procedures requires validation by the customer.

5.3. DNA AMPLIFICATION

- Ensure that the thermal cycler is powered on and the user has logged in.
- Work in the pre-PCR area.
- Use aerosol filter nuclease free pipette tips. Use a new tip for each DNA sample.
- It is not necessary to set up the PCR reaction on ice.
- Unless otherwise stated, “vortex” in this document refers to a process of vortexing the tube at maximum speed for 3-5 seconds while “spin down” refers to a process of centrifuging the tube at a minimum speed of 400g for 3-5 seconds.

1. Bring the DNA samples and ID CORE CONTROL 1 and ID CORE CONTROL 2 samples to room temperature.

   Note: A printed plate map with the position of all samples and controls in the run is recommended to ensure that the correct samples are dispensed in the appropriate wells.

2. Remove the HotStarTaq DNA polymerase from the freezer and the ID CORE XT PCR Master Mix from the 2ºC to 8ºC storage immediately before use.
3. Vortex and spin down both the HotStarTaq DNA polymerase and the ID CORE XT PCR Master Mix before use.
4. Calculate the required volumes of HotStarTaq DNA polymerase and ID CORE XT PCR Master Mix needed to prepare the ID CORE XT PCR reaction mix following the table below (all volumes in µl).

Number of Samples	1	8	16	24	32	40	48
ID CORE XT PCR Master Mix	22.5	180	360	540	720	900	1080
HotStarTaq DNA Polymerase (5 U/µl)	0.5	4	8	12	16	20	24

Note: The stated volumes already include an excess to account for potential volume lost during pipetting.

5. Prepare the ID CORE XT PCR reaction mix in a nuclease free 1.5 ml tube.
6. Return the HotStarTaq DNA polymerase to the freezer and the ID CORE XT PCR Master Mix to 2-8ºC storage immediately after use.
7. Vortex and spin down the ID CORE XT PCR reaction mix.

   Note: Once the ID CORE XT PCR reaction mix is prepared, the PCR program should start in ≤ 60 minutes.

8. Immediately dispense 20 µl per sample into the wells of a 96-well PCR plate.
9. Vortex and spin down the DNA samples and the ID CORE CONTROL 1 and the ID CORE CONTROL 2 samples.
10. Add 5 µl of sample DNA, ID CORE CONTROL 1, ID CORE CONTROL 2, and Negative Control to the corresponding wells. Mix gently by pipetting up and down three times.
11. Seal the plate with an adhesive film.

- **Work in the post-PCR area**

12. Spin down the PCR plate to collect the liquid at the bottom of the wells.
13. Verify that all the wells are properly sealed. Place the plate and the PCR compression pad on the thermal cycler block.
14. Close the thermal cycler lid and choose the pre-programmed ID XT PCR amplification program. Verify the pre-set temperature and the program settings before starting (details are listed in the table below).
15. Start the thermal cycler program.
16. Once the amplification step is finished, verify that ID XT PCR amplification program settings run without errors and save the Veriti Run Report.

	Temperature	Time	Cycles
Polymerase Activation	95°C	15:00	1
Denaturation	95°C	00:30	40 (ramp rate at 70%)
Annealing	60°C	00:30	
Extension	72°C	01:20	
Final Extension	72°C	07:00	1
Hold	4°C	∞	1
Amplification reaction volume: 25 µl			

Note: After PCR, amplification products can be kept at 2-8ºC or at -15ºC to -25ºC until the hybridization step.

Note: PCR products are a source of contamination and must remain in the post-PCR area.

Note: If the pre-programmed ID XT PCR amplification installed in the thermal cycler doesn´t match the program details listed in the table above, contact Grifols Technical Service.

5.4. HYBRIDIZATION

- Work in the post-PCR area.
- Ensure that the thermal cycler is powered on and the user has logged in.
- PCR products must be labeled immediately after hybridization. The process cannot be stopped after the hybridization step.
- Use a new aerosol filter nuclease free pipette tip for each sample.
- Unless otherwise stated, “vortex” in this document refers to a process of vortexing the tube at maximum speed for 3-5 seconds while “spin down” refers to a process of centrifuging the tube or plate at a minimum speed of 400g for 3-5 seconds.

1. Prior to the Hybridization reaction, turn on the Luminex 200 system and initialize the instrument.

   Note: Refer to the Luminex User’s Manual (xPONENT 3.1 Software User Manual) for instrument preparation and operation, including daily startup and calibration.

2. Set the Luminex 200 XYP instrument heater temperature at 52ºC and verify that the heater block is on the plate holder.
3. Remove the ID CORE XT Beads Master Mix from the 2-8ºC storage immediately before use.
4. Spin down the PCR plate to collect the amplification product at the bottom of the wells.
5. Vortex the ID CORE XT Beads Master Mix for 10-15 seconds.

   Note: Since the beads settle with time, the hybridization program should start in ≤30 minutes. Do not centrifuge the plate once the ID CORE XT Beads Master Mix has been dispensed to avoid bead sedimentation.

6. Dispense 46 µl of the ID CORE XT Beads Master Mix into each well of the hybridization plate (Bio-Rad). Avoid bubble formation during dispensing.

   Note: This is a critical processing step. Dispensation of half the recommended volume or less of BMM (<24 µl) could lead to incorrect genotypes.

7. Add 4 µl of PCR product into each well of the hybridization plate.
8. Mix gently by pipetting up and down three times. Avoid bubble formation during dispensing and pipetting.
9. Seal the plate with the Bio-Rad sealing film.
10. Verify that all the wells are properly sealed.
11. Place the plate and two compression pads on the thermal cycler block.
12. Close the thermal cycler lid and choose the pre-programmed ID XT HYB hybridization program. Verify the pre-set temperature of the lid and the program settings before starting (details are listed in the table below).
13. Start the thermal cycler program.

	Temperature	Time (min:sec)
Denaturation	95°C	02:00
Hybridization	52°C	30:00
Hold	52°C	∞

Note: If the pre-programmed ID XT HYB hybridization program installed in the thermal cycler doesn´t match the program details listed in the table above, contact Grifols Technical Service.

14. Return the ID CORE XT Beads Master Mix to the 2-8ºC storage.
15. During the hybridization step, prepare the labeling mix (see steps 1 to 6 of Section 5.5 LABELING) and create a new batch in the Luminex software (see steps 1 to 4 of Section 5.6 DATA ACQUISITION AND ANALYSIS).

5.5. LABELING

- Work in the post-PCR area.
- Labeled PCR products must be analyzed immediately after labeling. The process cannot be stopped after the labeling step.
- Use aerosol filter nuclease free pipette tips. Use a new tip for each sample.
- Unless otherwise stated, “vortex” in this document refers to a process of vortexing the tube at maximum speed for 3-5 seconds while “spin down” refers to a process of centrifuging the tube at a minimum speed of 400g for 3-5 seconds.

1. Remove the SAPE and the SAPE dilution buffer from the 2-8ºC storage immediately before use.
2. Vortex the SAPE and the SAPE dilution buffer and spin down the SAPE.
3. Calculate the required volumes of SAPE and SAPE dilution buffer needed to prepare the labeling mix following the table below (all volumes in µl).

Number of Samples	1	8	16	24	32	40	48
SAPE	4	32	64	96	128	160	192
SAPE Dilution Buffer	87	696	1392	2088	2784	3480	4176

Note: The stated volumes already include an excess to account for potential volume lost during pipetting.

4. Prepare the labeling mix in a 1.5 ml or a 15 ml tube.

   Note: Use the labeling mix in ≤35 minutes.

5. Return the SAPE and the SAPE dilution buffer to the 2-8ºC storage immediately after use.
6. Vortex the labeling mix. Keep it protected from light and at room temperature. After 30 minutes of hybridization (see Section 5.4 HYBRIDIZATION), at the 52ºC hold step, open the thermal cycler lid and carefully remove the compression pads and the sealing film, keeping the plate on the thermal cycler.
7. Dispense 80 µl of the labeling mix into each well of the hybridization plate and mix gently by pipetting up and down once.

   Note: The labeling mix should be dispensed to all samples in ≤5 minutes.

8. Seal the plate with a new Bio-Rad sealing film and place two compression pads on the plate. Close the thermal cycler lid and incubate the plate for 10 minutes at the 52ºC hold step.
9. After the labeling step, open the thermal cycler lid and remove the compression pads and the plate carefully from the thermal cycler.
10. Place the plate immediately on the Luminex, remove the sealing film and click “Run” to analyze the samples.

    Note: The elapsed time between removing the plate from the thermal cycler and placing it on the Luminex should not exceed 10 min.

11. Once the hybridization and labeling steps are finished, verify that the ID XT HYB hybridization program settings run without errors and save the Veriti DX Run Report.

5.6. DATA ACQUISITION AND ANALYSIS

- Refer to the Luminex User’s Manual (Luminex 200 User Manual and xPONENT 3.1. Software User Manual) for instrument preparation and operation, including probe adjustment, startup, calibration, verification, maintenance and shutdown procedures.
- Verify that the Luminex software data export configuration (“CSV Options” tab) is set at “By Analyte Name”, and that the boxes “Automatically export results CSV file when batch is complete”. “Use US regionalization format only”, “Include Advanced Statistics” and “Automatically convert the raw run files to CSV format for each well in the batch” is selected.
- Ensure the Luminex laser is warmed up, the plate heater block is placed in the XYP plate holder and the XYP temperature is 52ºC.

Data Acquisition

1. Select “Create a New Batch from an existing Protocol” in the batches tab and select the corresponding Luminex Template.

2. Enter a unique batch name. The name cannot contain characters other than letters (A-Z, a-z); numbers (0-9); hyphens (-), underscores (_) or spaces.
3. Create a batch by following the instructions that appear on the screen. A different batch is needed with each lot of reagents used. Therefore, if more than one lot of reagents are used in the same run, the Multibatch option will have to be selected and accordingly, multiple batches created. (For further instructions on creating batches and multibatches, refer to the *xPONENT 3.1 Software User Manual)*.
4. Select the corresponding positions in the Plate Layout and assign the samples as U ("Unknown").

   Enter the appropriate sample IDs carefully. It can be imported from a txt file by clicking "Import List".

   Note: To avoid incompatibility issues with the ID CORE XT ANALYSIS SOFTWARE, controls must be exactly named as follows: ID CORE CONTROL 1, ID CORE CONTROL 2 and Negative Control.
5. Click the "Eject" icon to eject the plate holder. Place the hybridization plate in the Luminex 200 XYP instrument heater block present on the plate holder and remove the sealing film.
6. Click the "Retract" icon. The samples are now ready to be analyzed.
7. Start the analysis process by clicking the "Run Batch" icon.
8. After the batch is complete, the data are exported as a Comma Separated Values (csv) file. This file is saved in a folder with the batch name entered previously in step 2.
9. The system can be shut down and turned off according to the Luminex User´s Manual at this point if it is not going to be used for the remainder of the day.

Data Analysis

Data Analysis is performed via the ID CORE XT ANALYSIS SOFTWARE. For more information on the data analysis procedure, refer to the ID CORE XT ANALYSIS SOFTWARE User Manual

For a run batch to be valid, the Negative Control and the positive controls (ID CORE CONTROL 1 and ID CORE CONTROL 2) within the batch run must be valid.

The invalid runs do not report polymorphism genotype, predicted allele genotype and predicted phenotype results for the samples included in that run. See troubleshooting section for more information.

The following table describes the valid and invalid results for the different types of samples analyzed.

Type of Sample	Expected Results
Negative Control	• O: VALID RUN. Negative Control average signal and individual amplicon signal are below the thresholds for the assay. No genomic DNA (gDNA) or amplicon contamination has been detected. Sample processing has been successful. • X: INVALID RUN. The average signal cannot be estimated due to low bead count; or the average signal for the Negative Control is above the threshold for the assay; and/or one or more amplicon signals are above the threshold. Sample processing is not reliable. No results will be provided for any of the samples analyzed. The entire batch must be retested.
Positive Controls	• O: VALID RUN. All ID CORE CONTROL 1 and ID CORE CONTROL 2 polymorphism genotype results are valid and in agreement with the expected pattern predefined in the ID CORE XT ANALYSIS SOFTWARE. Sample processing has been successful. • X: INVALID RUN. One or more of the polymorphism genotype results in ID CORE CONTROL 1 and/or ID CORE CONTROL 2 do not correspond with the expected pattern predefined in the ID CORE XT ANALYSIS SOFTWARE (low bead count, low average signal, low signal, indeterminate genotype or incorrect genotype). Sample processing is not reliable. No results will be provided for any of the samples analyzed. The entire batch must be retested.
DNA Sample	• O: VALID ANALYSIS. Polymorphism genotype, predicted allele genotype and predicted phenotype calls are displayed for all blood groups or no more than one blood group shows unknown as a result. Sample processing has been successful. • X: INVALID TEST. The sample is low average signal; either one or more of the polymorphism genotype results in the sample are low bead count, low signal or indeterminate genotype; or two or more blood groups are showing unknown as a result. No results will be provided for the sample analyzed. The sample must be retested.

The polymorphism genotype results follow the rules detailed in the following table (see Table 1 for more information):

General polymorphism genotype rules	Example
Polymorphism description (ISBT Gene Name)	*LU*:c.230A>G
The Rh and GYP rearrangements detected by ID CORE XT are described as “hybrid” genes	*GYP*. Hybrid
Homozygous for allele 1	AA
Heterozygous for allele 1 and 2	AG
Homozygous for allele 2	GG
Absent allele in an allele specific polymorphism	Absent
Present allele in an allele specific polymorphism (although homozygous or heterozygous status cannot be distinguished, the prediction of the allele genotype and the phenotype is not affected)	Present

Note: For the specific allele polymorphism RHCE:c.307T>C the results are reported as T or C instead of absent or present. Although heterozygous CT and homozygous CC results cannot be distinguished, both genotypes predict the same c+ phenotype.

The description of the predicted allele genotype results follows the rules detailed in the table below (see Table 1 for more information):

General predicted allele genotype rules	Example
Allele name description from the polymorphisms tested by ID CORE XT	*KEL*K_KPB_JSB*
Genotypes are written in italic	*GYPA*M*
Intronic sequences are indicated by "IVS_±_" and the polymorphism change in lower case	*JK*B_null(IVS5-1a)*
Alleles with a consensus name	*RHCE*ceAR*
Homozygous is represented by one allele	*DO*A*
Homozygous or hemizygous is not indicated	*RHCE*ce*
Heterozygous indicated by "," between each allele [$]	*RHCE*ce, RHCE*Ce*
Multiple changes in polymorphisms tested by ID CORE XT and without a consensus name are separated by "," and in brackets	*RHCE*ce[712G, 733G]*
Unknown	Highly unlikely prediction. The predicted allele genotype has not been described for the corresponding blood group.

$ The orientation (cis or trans) of the polymorphisms detected in heterozygosity cannot be determined by ID CORE XT. The most frequent predicted allele genotypes and phenotypes are reported; the alternative predicted allele genotype and/or phenotype are described in the corresponding "Note".

The following table summarizes the possible predicted phenotype results:

Predicted phenotype result	Meaning
+	Normal antigen expression
0	Undetectable antigen expression
Unknown	Highly unlikely prediction. The predicted phenotype has not been described for the corresponding antigen

In addition, ID CORE XT results include explanatory notes for some results outputs (see ID CORE XT ANALYSIS SOFTWARE User Manual for more information):

- The notes associated with the predicted allele genotype results describe the less frequent predicted allele genotype and potential implications in the corresponding phenotypes.
- The notes associated with the predicted phenotype results describe weak, partial or variable expression of the antigen.

6. TROUBLESHOOTING

Issue	Message	Cause	Solution
Invalid Run	Negative Control fails because average signal and/or amplicon signals are above the threshold	gDNA and/or amplicon contamination occurred	Retest the entire batch, ensuring that a new vial of nuclease-free molecular-grade water, aerosol-barrier tips, and a new tip for each sample is being used.
	Negative Control/ ID CORE CONTROL 1 and/or 2 fail because low bead count	The bead count of one or more polymorphisms is below the established threshold	Confirm the use of the provided Luminex template. Verify that Luminex probe is properly aligned. Confirm Luminex maintenance, calibration and verification following Luminex instructions. Retest the entire batch ensuring that all the processing steps described in the Package Insert are being followed.
	ID CORE CONTROL 1 and/or 2 fail because low average signal or low signal in one or more polymorphisms	The average signal or the signal of one or more particular polymorphisms is below the established threshold	Retest the entire batch ensuring that all the processing steps described in the Package Insert are being followed.
	ID CORE CONTROL 1 and/or 2 fail because indeterminate genotype	The genotype call for one or more polymorphisms cannot be determined because the discrimination value is outside the established limits	
	ID CORE CONTROL 1 and/or 2 fail because incorrect genotype	One or more polymorphism genotype results in ID CORE CONTROL 1 and/or ID CORE CONTROL 2 do not correspond with the expected pattern predefined in the ID CORE XT ANALYSIS SOFTWARE	
Invalid Test	A sample fails because low bead count	The bead count of one or more polymorphisms is below the established threshold	Retest the affected sample ensuring that all the processing steps described in the Package Insert are being followed.
	A sample fails because low average signal or low signal in one or more polymorphisms	The average signal or the signal of one or more particular polymorphisms is below the established threshold	
	A sample fails because indeterminate genotype	The genotype call for one or more polymorphisms cannot be determined because the discrimination value is outside the established limits	
	A sample fails because more two or more blood groups show unknown as a result	The allele genotype and phenotype cannot be predicted in two or more blood groups	
Unknown	Unknown	The allele genotype and phenotype cannot be predicted in one blood group	Another molecular method should be used to confirm the allele genotype and the phenotype should be predicted based on serology.

In invalid cases, if the issue persists, please contact Grifols Technical Service.

7. ANALYTICAL DATA

7.1. LIMIT OF DETECTION

The DNA concentration input range at which 100% of samples replicates resulted in correct polymorphism genotype and predicted phenotype results was determined by testing 20 replicates of serial DNA dilutions of ten representative DNA samples, which cover all genotypes with frequencies higher than 1% interrogated by ID CORE XT, using two reagent lots. The lowest and highest DNA concentrations providing 100% correct ID CORE XT results were determined to be 10 and 200 ng/µl, respectively.

7.2. PERFORMANCE CHARACTERISTICS

A. Accuracy study

An accuracy study was carried out to demonstrate the performance of ID CORE XT test to determine the polymorphism genotypes and predict the allele genotypes tested, as compared to Bi-Directional-Sequencing (BDS) and to predict the phenotypes tested, as compared to FDA licensed serology, when available, or BDS. The antigens characterized with licensed serology were: C (RH2), E (RH3), c (RH4), e (RH5), K (KEL1), k (KEL2), Jka (JK1), Jkb (JK2), Fya (FY1), Fyb (FY2), M (MNS1), N (MNS2), S (MNS3), and s (MNS4). The predicted phenotypes of the rest of the antigens interrogated by ID CORE XT were characterized with BDS: CW (RH8), V (RH10), hrS (RH19), VS (RH20), hrB (RH31), Kpa (KEL3), Kpb (KEL4), Jsa (KEL6), Jsb (KEL7), U (MNS5), Mia (MNS7), Dia (DI1), Dib (DI2), Doa (DO1), Dob (DO2), Hy (DO4), Joa (DO5), Coa (CO1), Cob (CO2), Yta (YT1), Ytb (YT2), Lua (LU1), and Lub (LU2).

The accuracy was determined using 1676 well characterized samples. The DNA samples were in a concentration range from 11.6 ng/µL to 244.2 ng/µL and a purity range (A260/A280) from 1.26 to 2.38.

The concordance % and one-sided 95% CI were calculated for polymorphism genotypes; predicted alleles and predicted phenotypes interrogated by ID CORE XT (see Tables 3, 4 and 5).

Table 3. Initial accuracy results (before discrepancy resolution) for the polymorphisms tested by ID CORE XT in comparison to BDS.

Blood Group System	Polymorphism	Polymorphism Genotype	Number of Samples	Concordance %	95% CI
Rh	*RHCE*:c.122A>G	AA	1143	100%	[0.997, 1.000]
		AG	28	100%	[0.899, 1.000]
		GG	1	100%	[0.050, 1.000]
	RHCE:c.307T>C	C	852	100%	[0.996, 1.000]
		T	317	99.68%	[0.985, 1.000]
	RHCE:c.335+3039ins	Absent	551	100%	[0.995, 1.000]
		Present	618	99.84%	[0.992, 1.000]
	RHCE:c.676G>C	676†	7	100%	[0.652, 1.000]
		CC	112	100%	[0.974, 1.000]
		GC	364	100%	[0.992, 1.000]
		GG	686	100%	[0.996, 1.000]
	RHCE:c.712A>G	712†	7	100%	[0.652, 1.000]
		712AA	1149	100%	[0.997, 1.000]
		712AG	13	100%	[0.794, 1.000]

Blood Group System	Polymorphism	Polymorphism Genotype	Number of Samples	Concordance %	95% CI
		712GG	6	100%	[0.607, 1.000]
	RHCE:c.733C>G	733†	7	100%	[0.652, 1.000]
		733CC	1004	100%	[0.997, 1.000]
		733CG	305	99.67%*	[0.985, 1.000]
		733GG	55	100%	[0.947, 1.000]
	RHCE:c.1006G>T	1006†	7	100%	[0.652, 1.000]
		1006GG	1151	100%	[0.997, 1.000]
		1006GT	14	100%	[0.807, 1.000]
		1006TT	6	100%	[0.607, 1.000]
	RHD-CE-D hybrid	Absent	1160	100%	[0.997, 1.000]
		Present	9	88.89%**	[0.571, 1.000]
Kell	*KEL*:c.578T>C	CC	998	100%	[0.997, 1.000]
		TC	98	100%	[0.970, 1.000]
		TT	69	100%	[0.958, 1.000]
	KEL:c.841T>C	CC	1142	100%	[0.997, 1.000]
		TC	20	100%	[0.861, 1.000]
		TT	3	100%	[0.368, 1.000]
	KEL:c.1790C>T	CC	23	100%	[0.878, 1.000]
		CT	63	100%	[0.954, 1.000]
		TT	1079	100%	[0.997, 1.000]
Kidd	*SLC14A1*:c.342-1G>A	GG	1161	100%	[0.997, 1.000]
		GA	5	100%	[0.549, 1.000]
		AA	4	100%	[0.473, 1.000]
	SLC14A1:c.838G>A	AA	316	100%	[0.991, 1.000]
		GA	472	100%	[0.994, 1.000]
		GG	375	100%	[0.992, 1.000]
	SLC14A1:c.871T>C	TT	1161	100%	[0.997, 1.000]
		TC	3	100%	[0.368, 1.000]
		CC	3	100%	[0.368, 1.000]
Duffy	*FY*:c.1-67T>C	CC	253	100%	[0.988, 1.000]
		TC	307	99.35%**	[0.980, 1.000]
		TT	717	100%	[0.996, 1.000]
	FY:c.125G>A	AA	560	100%	[0.995, 1.000]
		GA	430	99.53%**	[0.985, 1.000]
		GG	303	100%	[0.990, 1.000]
	FY:c.265C>T	CC	1138	100%	[0.997, 1.000]
		CT	22	100%	[0.873, 1.000]
		TT	3	100%	[0.368, 1.000]
MNS	*GYPA*:c.[59C>T]	CC	367	100%	[0.992, 1.000]
		CT	460	100%	[0.994, 1.000]
		TT	332	100%	[0.991, 1.000]
	GYPB:c.143T>C	143†	12	100%	[0.779, 1.000]
		CC	599	100%	[0.995, 1.000]
		TC	405	100%	[0.993, 1.000]
		TT	143	100%	[0.979, 1.000]
	GYPB:c.230C>T	230†	12	100%	[0.779, 1.000]
		CC	1140	100%	[0.997, 1.000]
		CT	12	100%	[0.779, 1.000]
		TT	5	100%	[0.549, 1.000]
	GYPB:c.270+5G>T	270+5†	12	100%	[0.779, 1.000]
		GG	1109	100%	[0.997, 1.000]
		GT	26	100%	[0.891, 1.000]
		TT	12	100%	[0.779, 1.000]

Blood Group System	Polymorphism	Polymorphism Genotype	Number of Samples	Concordance %	95% CI
	GYP. Hybrid	Absent	1149	99.74%**	[0.993, 1.000]
		Present	10	100%	[0.741, 1.000]
Diego	*DI*:c.2561T>C	CC	1153	100%	[0.997, 1.000]
		TC	17	100%	[0.838, 1.000]
		TT	8	100%	[0.688, 1.000]
Dombrock	*DO*:c.793A>G	AA	307	100%	[0.990, 1.000]
		AG	403	100%	[0.993, 1.000]
		GG	449	100%	[0.993, 1.000]
	DO:c.323G>T	GG	1103	100%	[0.997, 1.000]
		GT	43	100%	[0.933, 1.000]
		TT	13	100%	[0.794, 1.000]
	DO:c.350C>T	CC	1100	100%	[0.997, 1.000]
		CT	57	100%	[0.949, 1.000]
		TT	2	100%	[0.224, 1.000]
Colton	*CO*:c.134C>T	CC	1082	100%	[0.997, 1.000]
		CT	66	100%	[0.956, 1.000]
		TT	11	100%	[0.762, 1.000]
Cartwright	*YT*:c.1057C>A	AA	18	100%	[0.847, 1.000]
		CA	50	100%	[0.942, 1.000]
		CC	1091	100%	[0.997, 1.000]
Lutheran	*LU*:c.230A>G	AA	4	100%	[0.473, 1.000]
		GA	55	100%	[0.947, 1.000]
		GG	1100	99.91%	[0.996, 1.000]

†These polymorphism calls correspond to the absence of the *RH*CE or *GYPB* genes.
*These polymorphism genotypes provided 100% concordance with ID CORE XT after one sample exclusion (see Section C).
**These polymorphism genotypes provided 100% concordance with ID CORE XT after discrepancy resolution (see Section C).

Table 4. Initial accuracy results (before discrepancy resolution) for the predicted alleles tested by ID CORE XT in comparison to BDS.

Blood Group System	Predicted Allele	Number of samples	Concordance %	95% CI
Rh	*RHCE*ce*	482	99.79%	[0.990, 1.000]
	*RHCE*Ce*	598	99.67%	[0.990, 1.000]
	*RHCE*cE*	458	100%	[0.993, 1.000]
	*RHCE*CE*	29	100%	[0.902, 1.000]
	*RHCE*CeCW*	26	100%	[0.891, 1.000]
	*RHCE*ceCW*	0	N/A	N/A
	*RHCE*CECW*	0	N/A	N/A
	*RHCE*ceAR*	7	100%	[0.652, 1.000]
	*RHCE*ce[712G]*	5	100%	[0.549, 1.000]
	*RHCE*ce[733G]*	146	100%	[0.980, 1.000]
	*RHCE*ce[733G,1006T]*	3	100%	[0.368, 1.000]
	*RHCE*CeFV*	0	N/A	N/A
	*RHCE*Ce[712G,733G]*	0	N/A	N/A
	*RHCE*cEFM*	0	N/A	N/A
	*RHCE*cE[712G,733G]*	1	100%	[0.050, 1.000]
	RHCE-D[5, 7]-CE	7	100%	[0.652, 1.000]
	*RHD*r's-RHCE*ce[733G,1006T]*	8	100%	[0.688, 1.000]
	Unknown	2	50%*	[0.025, 1.000]
	*RHCE*Ce[733G]*	0	N/A	N/A

Blood Group System	Predicted Allele	Number of samples	Concordance %	95% CI
Kell	*KEL*K_KPB_JSB*	167	100%	[0.982, 1.000]
	*KEL*k_KPB_JSB*	1067	100%	[0.997, 1.000]
	*KEL*k_KPA_JSB*	23	100%	[0.878, 1.000]
	*KEL*k_KPB_JSA*	86	100%	[0.966, 1.000]
Kidd	*JK*A*	847	100%	[0.996, 1.000]
	*JK*B*	785	100%	[0.996, 1.000]
	*JK*B_null(IVS5-1a)*	2	100%	[0.224, 1.000]
	*JK*B_null(871C)*	2	100%	[0.224, 1.000]
	*JK*A_null(IVS5-1a)*	0	N/A	N/A
Duffy	*FY*A*	598	99.83%*	[0.992, 1.000]
	*FY*B*	646	99.54%*	[0.988, 1.000]
	*FY*B_GATA*	442	99.32%*	[0.983, 1.000]
	*FY*B[265T]_FY*X*	21	100%	[0.867, 1.000]
	*FY*A_GATA*	1	0%	[0.050, 1.000]
	*FY*A[265T]*	0	N/A	N/A
MNS(MN)	*GYPA*M*	827	100%	[0.996, 1.000]
	*GYPA*N*	792	100%	[0.996, 1.000]
MNS(Ss)	*GYPB*S*	512	99.80%*	[0.991, 1.000]
	*GYPB*s*	1004	99.80%*	[0.994, 1.000]
	*GYPB*S_null(230T)*	7	100%	[0.652, 1.000]
	*GYPB*S_null(IVS5+5t)*	38	97.37%*	[0.881, 1.000]
	*GYPB*deletion*	12	100%	[0.779, 1.000]
	GYP[140A]*	10	100%	[0.741, 1.000]
Diego	*DI*A*	25	100%	[0.887, 1.000]
	*DI*B*	1170	100%	[0.997, 1.000]
Dombrock	*DO*A*	688	100%	[0.996, 1.000]
	*DO*B*	825	100%	[0.996, 1.000]
	*DO*B_HY*	56	100%	[0.948, 1.000]
	*DO*A_JO*	59	100%	[0.950, 1.000]
Colton	*CO*A*	1148	100%	[0.997, 1.000]
	*CO*B*	77	100%	[0.962, 1.000]
Cartwright	*YT*A*	1141	100%	[0.997, 1.000]
	*YT*B*	68	100%	[0.957, 1.000]
Lutheran	*LU*A*	59	100%	[0.950, 1.000]
	*LU*B*	1155	99.91%	[0.996, 1.000]

*These alleles provided 100% concordance after discrepancy resolution (see Section C).

Table 5. Initial accuracy results (before discrepancy resolution) for the predicted phenotypes and each of the antigens tested by ID CORE XT in comparison to the Reference Methods: Bi-Directional-Sequencing (BDS) or FDA licensed serology (SER).

Blood Group System	Antigen	Predicted Phenotype	Number of Sample	NPA/PPA	95% CI	Reference Method
Rh	C (RH2)	Negative	516	100.00%	[0.994, 1.000]	SER
		Positive	548	99.82%	[0.991, 1.000]	
	E (RH3)	Negative	603	99.83%	[0.992, 1.000]	SER
		Positive	468	99.79%*	[0.990, 1.000]	
	c (RH4)	Negative	266	99.25%	[0.977, 1.000]	SER
		Positive	772	100.00%	[0.996, 1.000]	
	e (RH5)	Negative	108	100.00%	[0.973, 1.000]	SER
		Positive	911	100.00%	[0.997, 1.000]	
	CW (RH8)	Negative	1141	100%	[0.997, 1.000]	BDS
		Positive	26	100%	[0.891, 1.000]	

Blood Group System	Antigen	Predicted Phenotype	Number of Sample	NPA/PPA[3]	95% CI	Reference Method
		Unknown	2	N/A	[0.025, 1.000]	
	V (RH10)	Negative	1017	100%	[0.997, 1.000]	BDS
		Positive	149	100%	[0.980, 1.000]	
		Unknown	3	N/A	[0.135, 1.000]	
	hrS (RH19)	Negative	121	100%	[0.976, 1.000]	BDS
		Positive	1046	100%	[0.997, 1.000]	
		Unknown	2	N/A	[0.025, 1.000]	
	VS (RH20)	Negative	1012	100%	[0.997, 1.000]	BDS
		Positive	154	100%	[0.981, 1.000]	
		Unknown	3	N/A	[0.135, 1.000]	
	hrB (RH31)	Negative	202	100%	[0.985, 1.000]	BDS
		Positive	965	100%	[0.997, 1.000]	
		Unknown	2	N/A	[0.025, 1.000]	
Kell	K (KEL1)	Negative	935	100.00%	[0.997, 1.000]	SER
		Positive	118	100.00%	[0.975, 1.000]	
	k (KEL2)	Negative	68	100.00%	[0.957, 1.000]	SER
		Positive	120	100.00%	[0.975, 1.000]	
	Kpa (KEL3)	Negative	1142	100%	[0.997, 1.000]	BDS
		Positive	23	100%	[0.878, 1.000]	
	Kpb (KEL4)	Negative	3	100%	[0.368, 1.000]	BDS
		Positive	1162	100%	[0.997, 1.000]	
	Jsa (KEL6)	Negative	1079	100%	[0.997, 1.000]	BDS
		Positive	86	100%	[0.966, 1.000]	
	Jsb (KEL7)	Negative	23	100%	[0.878, 1.000]	BDS
		Positive	1142	100%	[0.997, 1.000]	
Kidd	Jka (JK1)	Negative	273	100.00%	[0.989, 1.000]	SER
		Positive	709	100.00%	[0.996, 1.000]	
	Jkb (JK2)	Negative	339	99.71%	[0.986, 1.000]	SER
		Positive	657	99.85%*	[0.993, 1.000]	
Duffy	Fya (FY1)	Negative	499	100.00%	[0.994, 1.000]	SER
		Positive	489	100.00%	[0.994, 1.000]	
	Fyb (FY2)	Negative	465	100.00%	[0.994, 1.000]	SER
		Positive	509	100.00%	[0.994, 1.000]	
MNS	M (MNS1)	Negative	279	100.00%	[0.989, 1.000]	SER
		Positive	699	99.86%*	[0.993, 1.000]	
	N (MNS2)	Negative	86	100.00%	[0.966, 1.000]	SER
		Positive	187	100.00%	[0.984, 1.000]	
	S (MNS3)	Negative	559	100.00%	[0.995, 1.000]	SER
		Positive	433	100.00%	[0.993, 1.000]	
	s (MNS4)	Negative	143	99.30%	[0.967, 1.000]	SER
		Positive	827	99.88%	[0.994, 1.000]	
	U (MNS5)	Negative	12	100%	[0.779, 1.000]	BDS
		Positive	1147	100%	[0.997, 1.000]	
	Mia (MNS7)	Negative	1149	99.74%**	[0.993, 1.000]	BDS
		Positive	10	100%	[0.741, 1.000]	
Diego	Dia (DI1)	Negative	1153	100%	[0.997, 1.000]	BDS
		Positive	25	100%	[0.887, 1.000]	
	Dib (DI2)	Negative	8	100%	[0.688, 1.000]	BDS
		Positive	1170	100%	[0.997, 1.000]	
Dombrock	Doa (DO1)	Negative	449	100%	[0.993, 1.000]	BDS
		Positive	710	100%	[0.996, 1.000]	
	Dob (DO2)	Negative	307	100%	[0.990, 1.000]	BDS
		Positive	852	100%	[0.996, 1.000]	
	Hy (DO4)	Negative	13	100%	[0.794, 1.000]	BDS
		Positive	1146	100%	[0.997, 1.000]	

Blood Group System	Antigen	Predicted Phenotype	Number of Sample	NPA/PPA§	95% CI	Reference Method
	Joa (DO5)	Negative	16	100%	[0.829, 1.000]	BDS
		Positive	1143	100%	[0.997, 1.000]	
Colton	Coa (CO1)	Negative	11	100%	[0.762, 1.000]	BDS
		Positive	1148	100%	[0.997, 1.000]	
	Cob (CO2)	Negative	1082	100%	[0.997, 1.000]	BDS
		Positive	77	100%	[0.962, 1.000]	
Cartwright	Yta (YT1)	Negative	18	100%	[0.847, 1.000]	BDS
		Positive	1141	100%	[0.997, 1.000]	
	Ytb (YT2)	Negative	1091	100%	[0.997, 1.000]	BDS
		Positive	68	100%	[0.957, 1.000]	
Lutheran	Lua (LU1)	Negative	1100	99.91%	[0.996, 1.000]	BDS
		Positive	59	100%	[0.950, 1.000]	
	Lub (LU2)	Negative	4	100%	[0.473, 1.000]	BDS
		Positive	1155	100%	[0.997, 1.000]	

§NPA/PPA are equivalent to Specificity/Sensitivity for BDS reference method.
*These antigens provided 100% concordance with ID CORE XT after one sample exclusion (see Section C).
**These antigens provided 100% concordance with ID CORE XT after discrepancy resolution (see Section C).

B. Clinical study: Method comparison study with a Comparable FDA approved product (genotyping kit predicating blood group antigen phenotypes), IVD licensed Serology and Bi-Directional-Sequencing.

A clinical method comparison study was carried out to validate the intended use of ID CORE XT testing genomic DNA extracted from whole blood samples collected in EDTA from 1026 blood donors in three reference blood centers in the United States and in 100 retrospective patients. The DNAs (with concentrations from 16.8 ng/µL to 191.2 ng/µL and purity (A260/A280) from 1.07 to 3.81 were processed with ID CORE XT, a Comparable FDA approved product (genotyping kit predicating blood group antigen phenotypes), and BDS. The antigens tested by a Comparable FDA approved product (genotyping kit predicating blood group antigen phenotypes) were compared to ID CORE XT predicted phenotypes, with the exception of Jkb (JK2) and Lub (LU2) antigens, which were compared to FDA-licensed serology tests (CE-mark serology test for Lub in patient samples). The antigens not tested by the Comparable FDA approved product (genotyping kit predicating blood group antigen phenotypes) and with no FDA-licensed serology available (i.e. CW (RH8), hrS (RH19), hrB (RH31), Mia (MNS7), Yta (YT1) and Ytb (YT2)) were compared to BDS results. All polymorphisms and predicted alleles interrogated by ID CORE XT were compared to BDS.

The valid run and valid test rates obtained in the study for ID CORE XT before repetition were 97.14% and 100%, respectively. After repetition, all ID CORE XT runs were valid and used in the study.

The concordance % and one-sided 95% CI were calculated for polymorphism genotypes, predicted alleles and predicted phenotypes interrogated by ID CORE XT (see Tables 6, 7 and 8).

Table 6. Initial results (before discrepancy resolution) for the polymorphisms tested by ID CORE XT in comparison to BDS.

Blood Group System	Polymorphism	Polymorphism Genotype	Number of Samples	Concordance %	95% CI
Rh	*RHCE:c.122A>G*	AA	1103	99.91%*	[0.996, 1.000]
		AG	23	100%	[0.878, 1.000]

Blood Group System	Polymorphism	Polymorphism Genotype	Number of Samples	Concordance %	95% CI
	RHCE:c.307T>C	C	854	99.77%**	[0.993, 1.000]
		T	272	100%	[0.989, 1.000]
	RHCE:c.335+3039ins109	Absent	501	100%	[0.994, 1.000]
		Present	625	100%	[0.995, 1.000]
	RHCE:c.676G>C	CC	26	100%	[0.891, 1.000]
		GC	192	100%	[0.985, 1.000]
		GG	908	99.89%**	[0.995, 1.000]
	RHCE:c.712A>G	AA	1125	100%	[0.997, 1.000]
		AG	1	100%	[0.050, 1.000]
	RHCE:c.733C>G	CC	1099	100%	[0.997, 1.000]
		CG	23	100%	[0.878, 1.000]
		GG	4	100%	[0.473, 1.000]
	RHCE:c.1006G>T	GG	1121	100%	[0.997, 1.000]
		GT	5	100%	[0.549, 1.000]
	RHD-CE-D hybrid	Absent	1122	100%	[0.997, 1.000]
		Present	4	100%	[0.473, 1.000]
Kell	*KEL*:c.578T>C	CC	1045	99.90%**	[0.995, 1.000]
		TC	81	98.77%**	[0.943, 0.999]
	KEL:c.841T>C	CC	1109	100%	[0.997, 1.000]
		TC	17	100%	[0.838, 1.000]
	KEL:c.1790C>T	CT	17	100%	[0.838, 1.000]
		TT	1109	100%	[0.997, 1.000]
Kidd	*SLC14A1*:c.342-1G>A	GG	1126	100%	[0.997, 1.000]
	SLC14A1:c.838G>A	AA	281	100%	[0.989, 1.000]
		GA	543	100%	[0.994, 1.000]
		GG	302	100%	[0.990, 1.000]
	SLC14A1:c.871T>C	TT	1126	100%	[0.997, 1.000]
Duffy	*FY*:c.1-67T>C	CC	40	100%	[0.928, 1.000]
		TC	32	93.75%**	[0.816, 0.989]
		TT	1054	100%	[0.997, 1.000]
	FY:c.125G>A	AA	430	100%	[0.993, 1.000]
		GA	510	99.8%*	[0.991, 1.000]
		GG	186	100%	[0.984, 1.000]
	FY:c.265C>T	CC	1093	100%	[0.997, 1.000]
		CT	32	100%	[0.911, 1.000]
		TT	1	100%	[0.050, 1.000]
MNS	*GYPA*:c.[59C>T]	CC	356	100%	[0.992, 1.000]
		CT	566	99.65*	[0.989, 0.999]
		TT	204	100%	[0.985, 1.000]
	GYPB:c.143T>C	CC	525	100%	[0.994, 1.000]
		TC	484	100%	[0.994, 1.000]
		TT	117	100%	[0.975, 1.000]
	GYPB:c.230C>T	CC	1126	100%	[0.997, 1.000]
	GYPB:c.270+5G>T	GG	1124	100%	[0.997, 1.000]
		GT	2	100%	[0.224, 1.000]
	GYP. Hybrid	Absent	1125	100%	[0.997, 1.000]
		Present	1	100%	[0.050, 1.000]
Diego	*DI*:c.2561T>C	CC	1123	100%	[0.997, 1.000]
		TC	2	100%	[0.224, 1.000]
		TT	1	100%	[0.050, 1.000]
Dombrock	*DO*:c.793A>G	AA	187	100%	[0.984, 1.000]
		AG	499	100%	[0.994, 1.000]
		GG	440	100%	[0.993, 1.000]
	DO:c.323G>T	GG	1118	100%	[0.997, 1.000]
		GT	7	100%	[0.652, 1.000]

Blood Group System	Polymorphism	Polymorphism Genotype	Number of Samples	Concordance %	95% CI
		TT	1	100%	[0.050, 1.000]
	DO:c.350C>T	CC	1119	100%	[0.997, 1.000]
		CT	7	100%	[0.652, 1.000]
Colton	*CO*:c.134C>T	CC	1042	100%	[0.997, 1.000]
		CT	82	100%	[0.964, 1.000]
		TT	2	100%	[0.224, 1.000]
Cartwright	*YT*:c.1057C>A	AA	2	100%	[0.224, 1.000]
		CA	100	100%	[0.970, 1.000]
		CC	1024	99.9%*	[0.995, 1.000]
Lutheran	*LU*:c.230A>G	AA	2	100%	[0.224, 1.000]
		GA	71	98.59%*	[0.935, 0.999]
		GG	1053	100%	[0.997, 1.000]

*These polymorphism genotypes provided 100% concordance with ID CORE XT after one sample exclusion (see Section C).
**These polymorphism genotypes provided 100% concordance with ID CORE XT after discrepancy resolution (see Section C).

Table 7. Initial results (before discrepancy resolution) for the alleles tested by ID CORE XT in comparison to BDS.

Blood Group System	Predicted Allele	Number of samples	Concordance %	95% CI
Rh	*RHCE*ce*	722	99.58%*	[0.989, 1.000]
	*RHCE*Ce*	618	99.35%**	[0.985, 1.000]
	*RHCE*cE*	217	100%	[0.986, 1.000]
	*RHCE*CE*	1	100%	[0.050, 1.000]
	*RHCE*CeCW*	23	100%	[0.878, 1.000]
	*RHCE*ceAR*	1	100%	[0.050, 1.000]
	*RHCE*ce[733G]*	21	100%	[0.867, 1.000]
	*RHCE*ce[733G,1006T]*	1	100%	[0.050, 1.000]
	*RHD*r's-RHCE*ce[733G,1006T]*	4	100%	[0.473, 1.000]
	*RHCE*Ce[733G]*	1	100%	[0.050, 1.000]
Kell	*KEL*K_KPB_JSB*	81	98.77%*	[0.943, 1.000]
	*KEL*k_KPB_JSB*	1125	99.82%*	[0.994, 1.000]
	*KEL*k_KPA_JSB*	17	100%	[0.838, 1.000]
	*KEL*k_KPB_JSA*	17	100%	[0.838, 1.000]
Kidd	*JK*A*	845	100%	[0.996, 1.000]
	*JK*B*	824	100%	[0.996, 1.000]
Duffy	*FY*A*	696	99.86%***	[0.993, 1.000]
	*FY*B*	874	99.66%**	[0.991, 1.000]
	*FY*B_GATA*	72	97.22%*	[0.915, 1.000]
	*FY*B[265T]_FY*X*	33	100%	[0.913, 1.000]
MNS (MN)	*GYPA*M*	922	99.78%	[0.993, 1.000]
	*GYPA*N*	770	99.74%	[0.992, 1.000]
MNS (Ss)	*GYPB*S*	599	100%	[0.995, 1.000]
	*GYPB*s*	1009	100%	[0.997, 1.000]
	*GYPB*S_null(IVS5+5t)*	2	100%	[0.224, 1.000]
	GYP[140A]*	1	100%	[0.050, 1.000]
Diego	*DI*A*	3	100%	[0.368, 1.000]
	*DI*B*	1125	100%	[0.997, 1.000]
Dombrock	*DO*A*	680	100%	[0.996, 1.000]
	*DO*B*	936	100%	[0.997, 1.000]
	*DO*B_HY*	8	100%	[0.688, 1.000]
	*DO*A_JO*	7	100%	[0.652, 1.000]
Colton	*CO*A*	1124	100%	[0.997, 1.000]

Blood Group System	Predicted Allele	Number of samples	Concordance %	95% CI
	*CO*B*	84	100%	[0.965, 1.000]
Cartwright	*YT*A*	1124	99.91%***	[0.996, 1.000]
	*YT*B*	102	100%	[0.971, 1.000]
Lutheran	*LU*A*	73	98.63%***	[0.937, 1.000]
	*LU*B*	1124	99.91%***	[0.996, 1.000]

*These alleles provided 100% concordance with ID CORE XT after discrepancy resolution (see Section C).
**These alleles provided 100% concordance with ID CORE XT after discrepancy resolution and one sample exclusion (see Section C).
***These alleles provided 100% concordance with ID CORE XT after one sample exclusion (see Section C).

Table 8. Initial results (before discrepancy resolution) for the predicted phenotypes and each of the antigens tested by ID CORE XT in comparison to the Reference Methods: a Comparable FDA approved product (genotyping kit predicating blood group antigen phenotypes) (Comparable product), Bi-Directional-Sequencing (BDS) or IVD licensed serology (SER).

Blood Group System	Antigen	Predicted Phenotype	Number of Sample	NPA/PPA[§]	95% CI	Reference Method
Rh	C (RH2)	Negative	496	100%	[0.994, 1.000]	Comparable product
		Positive	629	99.84%*	[0.992, 1.000]	
	E (RH3)	Negative	906	100%	[0.997, 1.000]	Comparable product
		Positive	219	100%	[0.986, 1.000]	
	c (RH4)	Negative	274	100%	[0.989, 1.000]	Comparable product
		Positive	851	100%	[0.996, 1.000]	
	e (RH5)	Negative	26	100%	[0.891, 1.000]	Comparable product
		Positive	1099	100%	[0.997, 1.000]	
	CW (RH8)	Negative	1103	99.91%*	[0.996, 1.000]	BDS
		Positive	23	100%	[0.878, 1.000]	
	V (RH10)	Possible Variant[†]	1	N/A	[0.050, 1.000]	Comparable product
		Negative	1103	100%	[0.997, 1.000]	
		Positive	21	100%	[0.867, 1.000]	
	hrS (RH19)	Negative	26	100%	[0.891, 1.000]	BDS
		Positive	1100	100%	[0.997, 1.000]	
	VS (RH20)	Possible Variant[†]	1	N/A	[0.050, 1.000]	Comparable product
		Negative	1099	100%	[0.997, 1.000]	
		Positive	25	96%*	[0.824, 1.000]	
	hrB (RH31)	Negative	33	100%	[0.913, 1.000]	BDS
		Positive	1093	100%	[0.997, 1.000]	
Kell	K (KEL1)	Negative	1044	100%	[0.997, 1.000]	Comparable product
		Positive	81	100%	[0.964, 1.000]	
	k (KEL2)	Positive	1125	100%	[0.997, 1.000]	Comparable product
	Kpa (KEL3)	Negative	1108	100%	[0.997, 1.000]	Comparable product
		Positive	17	100%	[0.838, 1.000]	
	Kpb (KEL4)	Positive	1125	100%	[0.997, 1.000]	Comparable product
	Jsa (KEL6)	Negative	1108	100%	[0.997, 1.000]	Comparable product
		Positive	17	100%	[0.838, 1.000]	
	Jsb (KEL7)	Positive	1125	100%	[0.997, 1.000]	Comparable product
Kidd	Jka (JK1)	Negative	281	100%	[0.989, 1.000]	Comparable product
		Positive	844	100%	[0.996, 1.000]	
	Jkb (JK2)	Negative	301	100%	[0.990, 1.000]	SER
		Positive	825	99.88%**	[0.994, 1.000]	
Duffy	Fya (FY1)	Negative	430	100%	[0.993, 1.000]	Comparable product
		Positive	695	100%	[0.996, 1.000]	

Blood Group System	Antigen	Predicted Phenotype	Number of Sample	NPA/PPA§	95% CI	Reference Method
	Fyb (FY2)	Negative	237	100%	[0.987, 1.000]	Comparable product
		Positive	888	100%	[0.997, 1.000]	
MNS	M (MNS1)	Negative	204	100%	[0.985, 1.000]	Comparable product
		Positive	921	99.89%	[0.995, 1.000]	
	N (MNS2)	Negative	356	100%	[0.992, 1.000]	Comparable product
		Positive	769	100%	[0.996, 1.000]	
	S (MNS3)	Negative	526	100%	[0.994, 1.000]	Comparable product
		Positive	599	100%	[0.995, 1.000]	
	s (MNS4)	Negative	117	100%	[0.975, 1.000]	Comparable product
		Positive	1008	100%	[0.997, 1.000]	
	U (MNS5)	Positive	1125	100%	[0.997, 1.000]	Comparable product
	Mia (MNS7)	Negative	1125	100%	[0.997, 1.000]	BDS
		Positive	1	100%	[0.050, 1.000]	
Diego	Dia (DI1)	Negative	1122	100%	[0.997, 1.000]	Comparable product
		Positive	3	100%	[0.368, 1.000]	
	Dib (DI2)	Negative	1	100%	[0.050, 1.000]	Comparable product
		Positive	1124	100%	[0.997, 1.000]	
Dombrock	Doa (DO1)	Negative	439	100%	[0.993, 1.000]	Comparable product
		Positive	686	100%	[0.996, 1.000]	
	Dob (DO2)	Negative	187	100%	[0.984, 1.000]	Comparable product
		Positive	938	100%	[0.997, 1.000]	
	Hy (DO4)	Negative	1	100%	[0.050, 1.000]	Comparable product
		Positive	1124	100%	[0.997, 1.000]	
	Joa (DO5)	Negative	1	100%	[0.050, 1.000]	Comparable product
		Positive	1124	100%	[0.997, 1.000]	
Colton	Coa (CO1)	Negative	2	100%	[0.224, 1.000]	Comparable product
		Positive	1123	100%	[0.997, 1.000]	
	Cob (CO2)	Negative	1041	100%	[0.997, 1.000]	Comparable product
		Positive	84	100%	[0.965, 1.000]	
Cartwright	Yta (YT1)	Negative	2	100%	[0.224, 1.000]	BDS
		Positive	1124	100%	[0.997, 1.000]	
	Ytb (YT2)	Negative	1024	99.90%*	[0.995, 1.000]	BDS
		Positive	102	100%	[0.971, 1.000]	
Lutheran	Lua (LU1)	Negative	1053	100%	[0.997, 1.000]	Comparable product
		Positive	72	100%	[0.959, 1.000]	
	Lub (LU2)	Negative	3	66.67%*	[0.135, 1.000]	SER
		Positive	1123	100%	[0.997, 1.000]	

§ NPA/PPA are equivalent to Specificity/Sensitivity for BDS reference method.
† Possible variant", the reference result, for V and VS antigens in one sample were positive and unknown by ID CORE XT respectively.
*These antigens provided 100% concordance after discrepancy resolution (see Section C).
** These antigens provided 100% concordance after one sample exclusion (see Section C).
NOTE: One sample was excluded from the comparison with the "Comparable product" due to an invalid result provided by this reference method ("No Typing Determined") caused by the presence of the variant *RHCE*:c.941T>C.

C. Discrepancy resolution and sample exclusion

- Accuracy study:

A total of seventeen (17) samples provided initial discrepant results. Two (2) of them were excluded from the final analysis and the other fifteen samples (15) were resolved in favour or against ID CORE XT. See the table below.

Blood Group System	Number of samples	Reference method	Discrepant Polymorphism*	Discrepant Antigen	ID CORE XT resolution	Rationale for the discrepancy
Rh	1	BDS	*RHD-CE-D* hybrid	N/A	In favour	Presence of variant *RHD-CE**IVS3+3046A>C
Rh	1	BDS	*RHCE*:c.733C>G	N/A	Excluded	"Invalid test" result due to presence of variant *KEL*:c.846G>C
Duffy	2	BDS	*FY*:c.1-67T>C	N/A	In favour	BDS sample mix-up
Duffy	2	BDS	*FY*:c.125G>A	N/A	In favour	BDS sample mix-up
MNS	3	BDS	*GYP*. Hybrid	Mia (MNS7)	In favour	Presence of variant *GYPA*:c.140C>A
Lutheran	1	BDS	*LU*:c.230A>G	Lua (LU1)	Against	ID CORE XT failure
Rh	1	BDS & Serology	*RHCE*:c.335+3039ins109	C (RH2)	Against	Presence of variant *RHCE*:c.335+3136A>G
Rh	1	BDS & Serology	*RHCE*:c.307T>C	c (RH4)	Against	Presence of variant *RHCE*:c.203G>A
Rh	1	Serology	N/A	E (RH3) c (RH4)	Against	Presence of variant *RHCE*:c.221G>A
Kidd	1	Serology	N/A	Jkb (JK2)	Against	Presence of variant *SLC14A1*:c.191G>A
MNS	1	Serology	N/A	s (MNS4)	Against	Presence of variant *GYPB*:c.137-43delAT
MNS	1	Serology	N/A	s (MNS4)	Against	Presence of variant *GYPB*:c.271-3delCAGGCAinsAAGCC
Rh Kidd MNS	1	Serology	N/A	E (RH3) Jkb (JK2) M (MNS1)	Excluded	Serology data was not confirmed in the donation

*Discrepant polymorphisms also provided discrepant predicted allele genotypes in comparison with BDS.

N/A. Not applicable

All discrepancies resolved against ID CORE XT in this study and the "Invalid test" result excluded from the analysis are described in Section 8 as "Assay Limitations" (Limitations 5, 9.1, 9.2, 9.3, 10.1, 10.2 and 10.3), with the exception of *LU*:c.230A>G related discrepancy (failure error).

- Clinical study:

A total of fourteen (14) samples provided initial discrepant results. Two (2) of them were excluded from the final analysis and the other twelve samples (12) were resolved in favour or against ID CORE XT. See the table below.

Blood Group System	Number of samples	Reference method	Discrepant polymorphism*	Discrepant antigen	ID CORE XT resolution	Rationale for Discrepancy
Rh	1	BDS	*RHCE*:c.307T>C	N/A	In favour	BDS sample mix-up
Rh	1	BDS	*RHCE*:c.676G>C	N/A	In favour	Presence of variant *RHCE:* c.801+219G>T
Rh	1	BDS	*RHCE*:c.307T>C	N/A	In favour	BDS sample mix-up

Blood Group System	Number of samples	Reference method	Discrepant polymorphism*	Discrepant antigen	ID CORE XT resolution	Rationale for Discrepancy
Kell	2	BDS	*KEL*:c.578T>C	N/A	In favour	BDS sample mix-up
Duffy	1	BDS	*FY*:c.1-67T>C	N/A	In favour	BDS sample contamination
Duffy	1	BDS	*FY*:c.1-67T>C	N/A	In favour	BDS sample contamination
MNS			*GYPA*:c.[59C>T]	N/A	In favour	
Rh	1	BDS	*RHCE*:c.122A>G	CW (RH8)	Excluded	DNA sample mix-up
Duffy			*FY*:c.125G>A	N/A		
MNS			*GYPA*:c.[59C>T]	N/A		
Cartwright			*YT*:c.1057C>A	Ytb (YT2)		
Lutheran			*LU*:c.230A>G	N/A		
MNS	1	BDS & Comparable product	*GYPA*:c.[59C>T]	M (MNS1)	Against	Presence of variant *GYPA*:c.38-66A>G
Rh	1	Comparable product	N/A	V (RH10)	In favour	Presence of *RHCE*Ce[733G]* allele
Rh	1	Comparable product	N/A	C (RH2)	In favour	Presence of *RHD*DIIIa* allele
Rh	1	Comparable product	N/A	VS (RH20)	In favour	Presence of *RHCE*ceAR* allele
Kidd	1	SER	N/A	Jkb (JK2)	Excluded	Deviation in serological procedure
Lutheran	1	SER	N/A	Lub (LU2)	In favour	Presence of variant *LU*:c.1615A>G

*Discrepant polymorphisms also provided discrepant predicted allele genotypes in comparison with BDS.

N/A. Not applicable

After discrepancy resolution ID CORE XT provided correct results for all the samples included in the study except for one sample (see Limitation 9.4 in Section 8 “Assay Limitation”).

D. Predicted alleles not tested in the performance studies (accuracy and clinical studies):

The following eight very rare alleles interrogated by ID CORE XT have not been represented by any sample in any of the performance studies carried out to validate ID CORE XT (see tables 4 and 7 above): *RHCE*ceCW* (Ref.1), *RHCE*CECW* (Ref.2), *RHCE*CeFV* (Ref.10), *RHCE*Ce[712G,733G]* (Ref.3), *RHCE*cEFM* (Ref.11), *JK*A_null(IVS5-1a)* (Ref.12), *FY*A_GATA* (Ref.13) and *FY*A[265T]* (Ref.14). However, based on (i) the information published in different bibliographic resources (Ref.1, 2, 3, 10, 11, 12, 13, 15, 16, 17 and 18) and (ii) the specific primers and probes design, those alleles can be correctly predicted by ID CORE XT.

In addition, the prediction of three of these alleles (*RHCE*CeFV*, *RHCE*Ce[712G,733G]* and *RHCE*cEFM*) by ID CORE XT are not based on all the associated polymorphisms described in the literature. The abovementioned bibliographic resources support the prediction of these alleles (see Table 2).

7.3. PRECISION

The precision is a measure of either the degree of repeatability and reproducibility of the analytical method under normal operating conditions.

The precision of the ID CORE XT assay was evaluated using a panel of ten representative DNA samples which cover all possible polymorphism genotypes with

frequencies higher than 1% interrogated by ID CORE XT.

A. Intermediate precision

The ID CORE XT assay demonstrates acceptable intermediate precision across operators (2), instruments (2), reagent lots (3), non-consecutive day/runs (6), and DNA concentrations (10, 20 and 200 ng/µl). The study results showed 100% of correct calls for polymorphism genotypes, predicted allele genotypes, and predicted phenotypes in all samples and replicates for each operator, instrument, day/run, reagent lot, and DNA concentration.

B. Reproducibility

The study showed that the ID CORE XT assay demonstrates acceptable reproducibility across external sites (3), operators (2 per site), and days (6 non-consecutive runs per operator and site). The valid run and valid test rates obtained before repetition were 97.22% and 100%, respectively. After repetition, the total ID CORE XT runs were valid and used in the study, which showed 100% of correct calls for polymorphism genotypes, predicted allele genotypes and predicted phenotypes in all samples and replicates for each site/instrument, operator and day/run.

Therefore, it can be concluded that the ID CORE XT test is highly reproducible.

7.4. ANALYTICAL SPECIFICITY: INTERFERING SUBSTANCES

The performance of ID CORE XT assay was not affected by any of the tested endogenous substances, exogenous substances and microorganisms.

A. Endogenous and exogenous substances

The impact of potential interfering substances present in specimens was assessed by analyzing three random EDTA anticoagulated whole blood samples. The endogenous interfering substances tested were intended to reproduce the states of hemolysis, icterus and lipemia in native blood specimens (>500 mg/dL of hemoglobin, >20 mg/dL of bilirubin and >3000 mg/dL of triglyceride-rich lipoprotein, respectively). The effect of total protein (>12 g/dL with >6 g/dL of albumin) was also evaluated.

As exogenous substances, and based on the intended use population of ID CORE XT, an antibiotic, a pain killer, anti-inflammatory drugs, blood thinners, a cholesterol lowering drug and an allergy medicine were also tested: ampicillin (152 µmol/L), acetaminophen (1324 µmol/L), ibuprofen (2425 µmol/L), aspirin (3.62 mmol/L), warfarin (32.5 µmol/L), heparin (3000 U/L), atorvastatin (600 µg/L) and diphenhydramine (19.6 µmol/L). Additionally, the potential co-extraction of ethanol (5%) and RNA (25% of the total nucleic acid concentration) were also evaluated as potential DNA sample contaminants.

The percent of correct calls was 100% for both polymorphism genotypes and predicted phenotypes across all samples, conditions and replicates.

B. Microorganisms

The ID CORE XT kit reagents were inoculated with 10^3-10^4 CFU/ml (CFU: Colony Forming Units) of the following bioburden strains to simulate microbial contamination: *Candida albicans, Aspergillus brasiliensis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Micrococcus luteus.* The percent of correct calls was 100% for both, polymorphism genotypes and predicted phenotypes, across all samples, conditions and replicates.

8. ASSAY LIMITATIONS

1. The predicted allele genotypes and phenotypes generated by the ID CORE XT test are inferred only from certain polymorphisms and certain alleles published in the scientific literature (see Table 1 in Principles of the Test).
2. The orientation (cis or trans) of the polymorphism detected as heterozygous cannot be determined by the assay. The most frequent predicted allele genotype and phenotype in the general population are reported and the alternative predicted allele genotype/s and/or phenotype/s are described in the corresponding Note. For the rare alleles without published frequency data, the predicted heterozygous genotype results reported by ID CORE XT are based on the frequency of the other allele in the general population, although the less likely genotype reported in the Note is also possible, e.g. "*RHCE*Ce, RHCE*cEFM* (24)". Note: (24) Also possible, although less likely: *RHCE*cE*, *RHCE*CeFV* or *RHCE*CE*, *RHCE*ce[712G*]. Possible phenotype: C+weak, e+weak or C+weak, e+weak and hrS-.
3. ID CORE XT assay cannot distinguish between the hemizygous and homozygous state for each of the alleles tested. In these cases, the reported genotype consists of a single allele.
4. The predicted allele genotype and phenotype with polymorphism combinations not described in the literature are reported by the ID CORE XT ANALYSIS SOFTWARE as "Unknown" results (see Troubleshooting Section).
5. For the specific allele polymorphism *RHCE*:c.307T>C detection, the genetic design is based on the described allele haplotype *RHCE*: (c.201A>G; c.203A>G; c.307C>T) associated with the expression of C antigen (Ref.7). Any variant of this haplotype, different than the one described, could provide an incorrect c.307C>T polymorphism genotype result.
6. The polymorphism interrogated by ID CORE XT to predict the Mia (MNS7) antigen, *GYPc.140A*, is shared by alleles *GYP.Mur*, *GYP.Hut* and *GYP.Bun* (Refs.15, 16, 17). The assay does not discriminate among them.
7. The phenotypes for the Rh antigens V (RH10), hrS (RH19), VS (RH20) and/or hrB (RH31) encoded by the alleles *RHCE*Ce[712G,733G]*, *RHCE*cE[712G,733G]*, *RHCE*CeFV*, *RHCE*cEFM* and *RHCE*Ce[733G]* interrogated by ID CORE XT that have not been reported in the literature are reported by the ID CORE XT ANALYSIS SOFTWARE as "Unknown" results.
8. Most of the Rh null phenotypes are generated by *RHCE* hybrids with *RHD* gene and related to the absence of expression of the RhCE antigens (Ref.6). The ID CORE XT test detects these *RHCE* hybrids as "*RHCE-D[5, 7]-CE*" allele genotype using the absence of signal from exons 5 and 7 of the *RHCE* gene. The prediction of absence of expression of the Rh antigens should always be confirmed by serology test, as it is described in the corresponding "Note".
9. False negative or invalid results may be generated by ID CORE XT due to rare mutations at primer or probe binding sites ("drop-out" artifacts). In such rare cases, this may lead to erroneous genotype and phenotype calls. The following are false negatives or invalid results incurred by ID CORE XT:
   9.1. Variant *RHCE*:c.335+3136a>g in intron 2 of the *RHCE*C* allele affects to the detection of polymorphism *RHCE*:c.335+3039ins109, leading to a false negative prediction of the C (RH2) antigen.
   9.2. Variant *GYPB*:c.137-43delAT in intron 3 of the *GYPB*s* allele affects to the detection of the *GYPB*:c.143T>C polymorphism leading to a false negative prediction of the s (MNS4) antigen.
   9.3. Variant *KEL*:c.846G>C in exon 8 of the *KEL*k_KPB_JSB* allele is described as rs8175993 and a global minor allele frequency (MAF) of 0.01 (Ref.18). This variant affects to the detection of the polymorphism KEL:c.841T>C which may lead to a false negative prediction of the Kpb (KEL4) antigen.
   9.4. Variant *GYPA*:c.38-66a>g in intron 2 of the *GYPA*M* allele is described as rs535847209 and a global minor allele frequency (MAF) of 0.0002 (Ref.18). This variant affects to the detection of *GYPA*:c.[59C>T] polymorphism leading to a false negative prediction of the M (MNS1) antigen.
10. False positive results may be generated by ID CORE XT due to null alleles not

detected in the gene tested or variants in other regulatory genes, or post transcriptional events, or epigenetic events not tested by the assay. In these cases, the predicted phenotype may differ from the phenotype detected by serology. The following are false positives incurred by ID CORE XT:

10.1. Variant *RHCE*:c.221G>A in exon 2 of the *RHCE*cE221A* allele, is associated with the absence of expression of the E (Rh3) and c (RH4) antigens (Ref.8).

10.2. Variant SLC14A1:c.191G>A in exon 4 of the JK*B_null(191A) allele is described as rs114362217 and a global minor allele frequency (MAF) of 0.0004 (Ref.18). This variant was described in African Americans with a rare occurrence and associated with the absence of expression of the Jkb (JK2) antigen (Ref.9).

10.3. Splicing site variant GYPB:c.271-3delCAGGCAinsAAGCC in intron 5 of the unreported rare allele GYPB*s_(IVS5-3delCAGGCAinsAAGCC) is associated with the absence of expression of the s (MNS4) antigen.

11. The ID CORE XT ANALYSIS SOFTWARE allows the association of only one ID CORE XT reagent lot, one ID CORE CONTROL lot, and one enzyme lot per Luminex run (one association of reagent lots and enzyme lot per .csv file generated by the Luminex). If two or more ID CORE XT reagent lots, ID CORE CONTROL lots, or enzyme lots are associated with a group of samples, each lot needs to be run independently including the Positive and Negative Controls.
12. Each batch of samples per reagent lot must be tested with one replicate of the two positive control samples included in ID CORE CONTROL (ID CORE CONTROL 1 and ID CORE CONTROL 2) and one replicate of the Negative Control (nuclease-free molecular-grade water) at the end of the batch. Each batch of samples cannot be tested with more than one replicate of any ID CORE CONTROL (ID CORE CONTROL 1 and ID CORE CONTROL 2).

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19. TRADEMARKS

The following product names and any registered and unregistered trademarks mentioned in this document are used for identification purposes only and remain the exclusive property of their respective owners.

ID CORE XT, ID CORE XT ANALYSIS SOFTWARE ID CORE CONTROL	Progenika Biopharma S.A.
Microseal, Multiplate	Bio-Rad Inc.
MicroAmp, Veriti	Applera HS Corporation
HotStarTaq DNA Polymerase, QIAamp, QIAsymphony	QIAGEN
Luminex 200, xPONENT, xMAP	Luminex Corporation

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21. SYMBOLS USED IN PACKAGING

Consult Instructions for Use

Manufacturer

Contains Sufficient for <n> Tests

Temperature Limitation

Catalog Number

Use by Date: YYYY-MM-DD

Batch code

In vitro diagnostic medical device

22. CUSTOMER AND TECHNICAL SERVICES

Contact Grifols Customer Service to order additional reagents. Contact Grifols Technical Service for comments or questions on ID CORE XT procedures, equipment, reagents, or data analysis.

Grifols Customer Service:
Telephone: (888) 244-7667
Or: (510) 923-5100
E-mail: DxSCMCustomer.Service@grifols.com

Grifols Technical Service:
Telephone: (800) 452-6877
E-mail: service.americas@grifols.com

23. ANNEX I. ASSAY GUIDELINES

The complete Package Insert needs to be read before proceeding with sample analyses.

A. PRE-PCR AREA

DNA AMPLIFICATION

1. Bring the **DNA samples** and **ID CORE CONTROL 1** and **ID CORE CONTROL 2** to room temperature.

   ***Note**: A printed plate map with the position of all samples and controls in the run is recomme nded to ensure that the correct samples are dispensed in the appropriate wells.*

2. Remove the **HotStarTaq DNA polymerase** from the freezer and the **ID CORE XT PCR Master Mix** from the 2ºC to 8ºC storage immediately before use.
3. Vortex and spin down both reagents.
4. Prepare the **ID CORE XT PCR reaction mix** following the table below (all volumes in µl).

Number of Samples	1	8	16	24	32	40	48
ID CORE XT PCR Master Mix	22.5	180	360	540	720	900	1080
HotStarTaq DNA Polymerase (5 U/µl)	0.5	4	8	12	16	20	24

5. Store reagents immediately after use.
6. Vortex and spin down the ID CORE XT PCR reaction mix.

   ***Note:** Once the ID CORE XT PCR reaction mix is prepared, the PCR program should start in ≤ 60 minutes.*

7. Immediately dispense **20 µl** per sample into the wells of a 96-well PCR plate.
8. Vortex and spin down the DNA samples and ID CORE CONTROL 1 and the ID CORE CONTROL 2 samples.
9. Add **5 µL** of sample DNA, ID CORE CONTROL 1, ID CORE CONTROL 2, and Negative Control to the corresponding wells. Mix gently by pipetting up and down three times.
10. Seal the plate with adhesive film and follow the guidelines from the Post-PCR Area DNA AMPLIFICATION section.

B. POST-PCR AREA

1. Spin down the PCR plate.
2. Place the plate and the PCR compression pad on the thermal cycler block.
3. Close the thermal cycler lid, verify and start the **ID XT PCR** amplification program.
4. Once the amplification step is finished, verify and save the Veriti Run Report.

HYBRIDIZATION

Note: *Refer to the Luminex User's Manual (xPONENT 3.1 Software User Manual) for instrument preparation and operation, including daily startup and calibration.*

5. Prior to the Hybridization reaction, turn on the Luminex 200 system
6. Set the Luminex 200 XYP heater temperature at **52ºC** and verify that the heater block is on the plate holder.
7. Remove the **ID CORE XT Beads Master Mix** from the 2ºC to 8ºC storage immediately before use.
8. Spin down the PCR plate.
9. Vortex the ID CORE XT Beads Master Mix for **10-15** seconds.

   Note: *Since the beads settle with time, the hybridization program should start in ≤30 minutes. Do not centrifuge the plate once the ID CORE XT Beads Master Mix has been dispensed to avoid bead sedimentation.*

10. Dispense **46 µl** of the ID CORE XT Beads Master Mix into each well of the Bio-Rad hybridization plate.

    Note: *This is a critical processing step. Dispensation of half the recommended volume or less of BMM (< 24 µl) could lead to incorrect genotypes.*

11. Add **4 µl** of each PCR product into each well of the hybridization plate. Mix gently by pipetting up and down 3 times.
12. Seal the plate with the Bio-Rad sealing film.
13. Place the plate and two compression pads on the thermal cycler block.
14. Close the thermal cycler lid, verify and start the **ID XT HYB** hybridization program.
15. Return the ID CORE XT Beads Master Mix to the 2ºC to 8ºC storage.
16. During the hybridization step prepare the **labeling mix** and **Create a New Batch** in the Luminex software.

LABELING

17. Remove the **SAPE** and **SAPE Dilution Buffer** from the 2ºC to 8ºC storage immediately before use.
18. Vortex the SAPE and SAPE Dilution Buffer and spin down the SAPE.
19. Prepare the labeling mix following the table below (all volumes in µl). Use the labeling mix in ≤ 35 minutes.

Number of Samples	1	8	16	24	32	40	48
SAPE	4	32	64	96	128	160	192
SAPE Dilution Buffer	87	696	1392	2088	2784	3480	4176

20. Return the SAPE and SAPE dilution buffer to the 2ºC to 8ºC storage immediately after use.
21. Vortex the labeling mix. Keep it protected from light and at room temperature.
22. After 30 minutes of hybridization, at the 52ºC hold step, open the thermal cycler lid and carefully remove the compression pads and the sealing film, keeping the plate on the thermal cycler.
23. Dispense 80 µl of the labeling mix into each well of the hybridization plate and mix gently by pipetting up and down once. Note: The labeling mix should be dispensed to all samples in ≤5 minutes.
24. Seal the plate with a Bio-Rad sealing film and place two compression pads or one compression mat on the plate. Close the thermal cycler lid and incubate the plate for 10 minutes at the 52ºC hold step.
25. After the hybridization and labeling steps, open the thermal cycler lid and remove the compression pads and the plate carefully from the thermal cycler. Verify and save the Veriti DX Run Report.
26. Place the plate on the Luminex, remove the sealing film and click Run to analyze the samples.

    Note: *The time between removing the labelled plate from the thermal Cycler and placing it on Luminex should not exceed 10 min.*