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SUMMARY OF SAFETY AND EFFECTIVENESS DATA

Product: ID CORE XT™

1. General Information

Device Trade Name:	ID CORE XT™
Device Product Code:	PEP
Applicant Name and Address:	Progenika Biopharma S.A. Parque Tecnológico de Bizkaia Ibaizabal bidea, Edificio 504 C.P. 48160, Derio – Bizkaia (Spain)
Establishment Registration Number:	3006413195
Premarket Approval Application (PMA) Number:	BP170154
Date of Panel Recommendation:	Not Applicable
Office's Signatory Authority	Nicole Verdun, M.D. Director, OBRR/CBER

- □ **I concur with the summary review.**
- □ **I concur with the summary review and include a separate review to add further analysis.**
- □ **I do not concur with the summary review and include a separate review.**

Date of FDA Notice of Approval: October 11, 2018

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE)

Review memos from the following reviewers were used in developing the SSE:

Discipline	**Reviewer Names**
Chemistry/Manufacturing/Controls (CMC)	Zhugong "Jason" Liu Jeremy Wally Meihong Liu Darcel Bigelow
Analytical Studies and Clinical Studies	Zhugong "Jason" Liu Meihong Liu Linye Song Zhen Jiang
Software	Darcel Bigelow
Pre-approval inspection	Zhugong "Jason" Liu Teresita Mercado Travis S Bradley
Bioresearch Monitoring Inspection (BIMO)	Haecin Chun
Statistician	Linye Song Zhen Jiang
Product and Promotional Labeling	Zhugong "Jason" Liu Meihong Liu Dana Jones

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*rsRHCE*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)
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:c.[59C>T]* *GYPB:c.143T>C* *GYPB:c.230C>T* *GYPB:c.270+5G>T* *GYP.hybrid*	*GYPA*M* *GYPA*N* *GYPB*S* *GYPB*s* *GYPB*S_null(230T)*	M (MNS1) N (MNS2) S (MNS3) s (MNS4) U (MNS5)

		*GYPB*S_null(IVS5+5t)* *GYP*[140A]* *GYPB*deletion*	Mia (MNS7)
Diego	*DI:c.2561T>C*	*DI*A* *DI*B*	Dia (DI1) Dib (DI2)
Dombrock	*DO:c.323G>T* *DO:c.350C>T* *DO:c.793A>G*	*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)

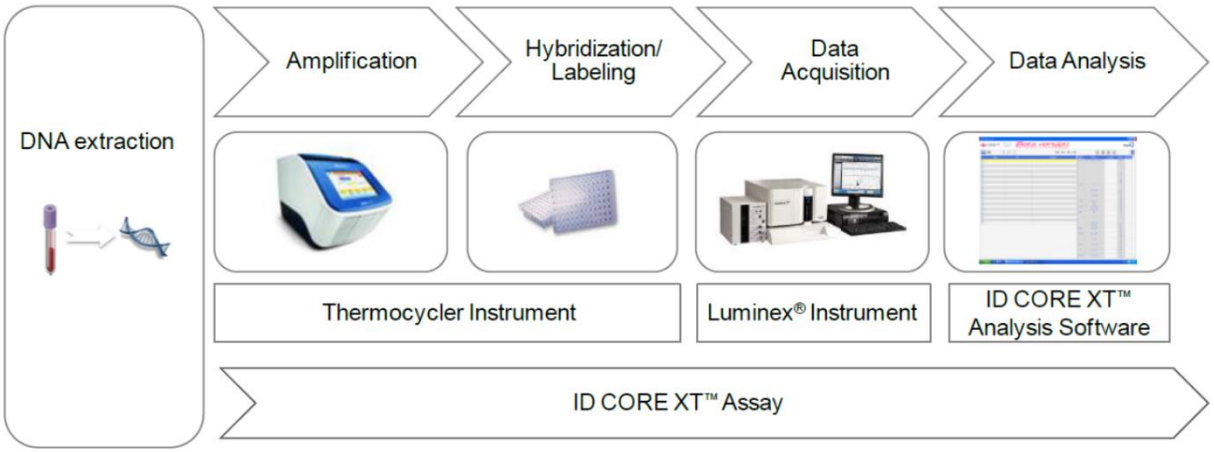
3. Description:

3.1 Device Description

ID CORE XT™ is a qualitative, polymerase chain reaction (PCR) and hybridization-based genotyping test that provides results for 29 genetic polymorphisms, 53 alleles and 37 antigens of ten blood group systems. The device consists of reagents and analysis software. The test uses PCR to amplify the target DNA sequence encoding blood group antigens. The assay is performed using 96-well plates, where the analysis of all the genetic variants tested per sample is performed in one single well. The assay starts with human genomic DNA and consists of the following major steps:

- Amplification
- Hybridization/labeling
- Data Acquisition
- Data Analysis

The following is a schematic representation of these assay steps:



Genomic DNA is extracted from human EDTA anticoagulated whole blood. Multiplex PCR is used to amplify and biotinylate the regions of genomic DNA containing the polymorphisms targeted by this test.

PCR products are then denatured and hybridized to oligonucleotide probes on the surface of each (b) (4) bead. Each probe is coupled to a unique color-coded bead. Hybridized DNA fragments are labeled with fluorochrome via binding of Streptavidin-PE (SAPE) to the biotin attached to the amplified DNA.

The data acquisition step is performed with a Luminex 200 system. This system acts as a flow cytometer and has two lasers. The first laser identifies the internal dye mixture of each bead; the second laser detects the fluorescent signal intensity coming from the fluorochrome attached to the biotinylated DNA.

In the data analysis step, raw data (Median fluorescence intensity (MFI)) from the Luminex system are analyzed by the ID CORE XT ANALYSIS SOFTWARE to determine the polymorphism genotypes. Based on the polymorphisms, the software predicts and reports allele genotype and phenotype for each HEA interrogated by the test.

3.2. Kit Configurations and Components

The ID CORE XT consists of four reagents and a compact disc supplied by Progenika Biopharma S.A. (Progenika). The reagents provided in one kit are sufficient for 48 tests. An information sheet is also included to provides the part number and lot number (barcode) for each kit component.

The ID CORE CONTROL is required for quality control of the ID CORE XT assay and is sold separately. This control kit consists of two reagent components (ID CORE CONTROL 1 and ID CORE CONTROL 2, one vial each) supplied by Progenika in a single package.

3.2.1. Reagent Components

The ID CORE XT kit consists of the following reagent components:

ID CORE XT PCR Master Mix
ID CORE XT Beads Master Mix
Streptavidin phycoerythrin (SAPE)
SAPE Dilution Buffer

All reagents are provided ready for use.

3.2.2. Compact disk

The compact disk contains the following information:

ID CORE XT ANALYSIS SOFTWARE
ID CORE XT Package Insert
ID CORE XT ANALYSIS SOFTWARE User Manual
ID CORE XT Luminex template

The ID CORE XT ANALYSIS SOFTWARE is a software program used to interpret Luminex 200 system MFI readings and convert them into polymorphism genotypes, predicted allele

genotypes and predicted phenotypes. In addition, the run validity is also determined by the software based on the results provided by the three process controls (Negative Control, ID CORE CONTROL 1, and ID CORE CONTROL 2). The software provides all the results as a pdf report summarizing the outcomes for each test sample.

3.2.3. External Assay Controls

Use of the ID CORE XT requires two positive controls and one negative control (not provided as part of ID CORE XT kit) to determine run validity. The ID CORE CONTROL kit, which is purchased separately from the ID CORE XT kit, consists of 2 vials of (b) (4) DNA plasmid pools designed as positive controls for the correct detection of the polymorphisms assayed by the ID CORE XT. The negative control is nuclease free molecular-grade water and is included in each assay run to check for potential DNA contamination.

3. 2.4. Materials Required (but not provided)

- DNA extraction kit
- HotStarTaq DNA Polymerase, QIAGEN (700410)
- Nuclease free molecular-grade water
- 96-well reaction plates
- Pipettes
- Disposable aerosol filter pipette tips
- Nuclease-free tubes
- Plate centrifuge
- Micro centrifuge
- Vortex mixer
- Spectrophotometer
- Veriti Dx 96-Well Thermal Cycler
- Luminex 200
- Luminex Calibrators and Controls

4. Test Procedure

4.1. Specimen Collection, Preparation, and Storage

4.1.1. Collecting Specimens

The ID CORE XT assay is performed on human genomic DNA extracted from whole blood collected with EDTA as the anticoagulant. Three different types of EDTA anticoagulant ((b) (4)-EDTA, (b) (4)-EDTA or (b) (4)-EDTA) have been validated for use with the ID CORE XT.

4.1.2. Storing Specimens

EDTA anti-coagulated whole blood samples are stable for storage at 2-8 °C for (b) (4) before DNA extraction. Extracted DNA samples can be stored frozen at -15 ºC or below for at least (b) (4) before being used in the ID CORE XT assay.

4.2. Assay Processing Steps

The assay begins with the extraction of the DNA from whole blood samples using DNA extraction kits recommended in the package insert. This is followed by an amplification step, where ID CORE XT uses the PCR technique to obtain large amounts of the DNA regions encoding HEAs and label them with biotin. The ID CORE XT PCR Master Mix component contains specific primers designed for the PCR amplification of these regions.

Amplified DNA sequences containing the genetic polymorphisms to be tested are then denatured and hybridized to oligonucleotide probes coupled to color-coded beads ((b) (4)), included in the ID CORE XT Beads Master Mix component. In most cases, two probes are needed for the analysis of each polymorphism, one complementary to the sequence of the common allele and the other specific for the variant allele. Several polymorphisms are genotyped using allele-specific primers, designed for the selective amplification of each allele. If the allele is not present, amplification will not occur. Hybridized DNA is labeled with a fluorescent conjugate (SAPE component diluted in the SAPE Dilution Buffer) with high binding affinity for the biotin incorporated into the DNA in the amplification step.

In the data acquisition step, the fluorescent signal associated with each specific probe is detected with a Luminex 200 system. Briefly, the Luminex 200 acts as a flow cytometer, allowing the analysis of the mixture of different oligonucleotide probe-coupled beads one by one. One of the two laser identifies the internal dye mixture of each bead. The other laser detects the fluorescence emission intensity coming from the fluorescent conjugate attached to the biotinylated DNA, bound to the probes on the surface of each bead.

In the final step, raw data from the Luminex System using xPONENT 3.1 Software (csv. files containing the MFI value for each bead type, among other data) is processed by the ID CORE XT ANALYSIS SOFTWARE. This software converts the MFI values into polymorphism genotype. Once all polymorphism genotypes have been assigned, the software uses a cross-reference table to predict the allele genotypes and phenotypes associated with each blood group system. Finally, the software generates a pdf report summarizing the outcomes for each test sample.

4.3. Quality Control

The ID CORE CONTROL kit and Negative Control (nuclease-free molecular-grade water) are used for quality control of the ID CORE XT assay. Each run 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. For a run to be considered valid, the ID CORE XT ANALYSIS SOFTWARE confirms that:

a. The Negative control sample provides a minimum number of individual bead counts per bead type, an average signal (xMFI) below the pre-established threshold and individual signals per amplicon below the established thresholds.

b. ID CORE CONTROL 1 and ID CORE CONTROL 2 samples show a minimum number of individual bead counts per bead type, an average signal (xMFI) equal or above the pre-established threshold, a minimum MFI value for each probe, as well as 100% correct calls for the expected polymorphism genotypes.

4.4. Interpretation of Results

Assay validity and test results are determined by the ID CORE XT ANALYSIS SOFTWARE. There were two levels of validity check for the ID CORE XT assay. The first level is at the run level, and the software determines whether a run is valid based on data from positive and negative controls. If either the positive or negative controls fail during a run, the software will provide an "Invalid Run" message and report the reason for the failure. No genotype or phenotype results will be provided for any test samples in invalid runs.

The second validity check is at the test level. Results for a sample are deemed invalid when at least one of the polymorphisms cannot be called due to "Indeterminate genotype", "Low signal intensity", or "Low bead count". "Indeterminate genotype" is called when one or more polymorphisms cannot be determined because the discrimination value is outside the established limits. In addition, a test is deemed invalid if more than one "Unknown (UN)" message (i.e., a combination of polymorphisms is not included in the ID CORE XT ANALYSIS SOFTWARE) is generated for one blood group system. When the conditions for valid test are not fulfilled, the software will display an "Invalid Test" message.

Three result types are provided for each valid sample: a "Polymorphism Genotype Result" for each tested polymorphism, a "Predicted Allele Genotype Result" for each tested allele genotype, and a "Predicted Phenotype Result" for each antigen. The following table summarizes the possible predicted phenotype results:

Table 2: Predicated phenotype results reported by ID CORE XT

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, explanatory notes are provided for some predicted allele genotypes and predicted phenotypes, based on the literature. For example, a note may explain that a weak expression of an antigen by serology is detected and reported by the ID CORE XT as a positive antigen phenotype.

5. Limitations of the Test

The limitations of the ID CORE XT are described in the Package Insert.

- 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.
- 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 test report explanatory 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 explanatory note is also possible.

- 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.
- 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.
- 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.
- 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*. The assay does not differentiate these alleles.
- 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.
- 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. 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".
- 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:
    - Variant *RHCE*:c.335+3136a>g in intron 2 of the *RHCE*C* allele affects the detection of polymorphism *RHCE*:c.335+3039ins109, leading to a false negative prediction of the C (RH2) antigen.
    - Variant *GYPB*:c.137-43delAT in intron 3 of the *GYPB*s* allele affects the detection of the *GYPB*:c.143T>C polymorphism leading to a false negative prediction of the s (MNS4) antigen.
    - 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. This variant affects the detection of the polymorphism KEL:c.841T>C which may lead to a false negative prediction of the Kpb (KEL4) antigen.
    - 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. This variant affects the detection of *GYPA*:c.[59C>T] polymorphism leading to a false negative prediction of the M (MNS1) antigen.

- 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:
    - 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.
    - 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. This variant was described in African Americans with a rare occurrence and associated with the absence of expression of the Jkb (JK2) antigen.
    - 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.
- 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.
- 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).

6. Warnings and Cautions

Relevant warning and caution statements are included in the ID CORE XT Package Insert.

7. Marketing History

The ID CORE XT device was CE-marked in May, 2014, and subsequently registered in Australia, Canada, Saudi Arabia, Thailand, Korea, Brazil, Mexico, Macedonia, and Egypt. According to the manufacturer, ID CORE XT has not been withdrawn from the market in any country for any reason related to the safety or effectiveness of the device. The “Research Use Only” product has been available in the US since 2010.

8. Potential Adverse Effects of the Device on Health

DNA analysis-based techniques such as ID CORE XT show some general limitations in the prediction of the phenotypes of the HEAs. The main limitation of the genotyping tests is the presence of rare mutations at primer or probe binding sites leading to false negative results. In addition, the genotype may not correlate with the phenotype detected by serology due to the

presence of rare null alleles not tested by the assay, leading to false positive phenotype results. This test involves complex technology and users need to be adequately trained on its use.

These limitations may cause false positive or false negative typing results for donor or patient samples, which could lead to adverse events of different severity.

- In the case of a donor unit with a false negative result, a patient with a clinically significant antibody could be transfused with antigen positive blood which may result in a hemolytic transfusion reaction.
- A donor unit with a false positive result would not be chosen for patient transfusion, which could lead to delays in obtaining compatible blood for transfusion.
- A false negative result in a patient sample could lead to an erroneous conclusion that an antigen negative unit is required for the patient, and delays in obtaining compatible blood.
- A false positive result in a patient sample may lead to the transfusion of blood that has the antigen the patient does not have. This may lead to alloimunization in the patient complicating future transfusions, or a transfusion reaction if the patient has a clinically significant antibody.

The risk of incorrect results is minimized by following the procedures and instructions outlined in the Package Insert. Progenika has also included a list of known limitations of the assay in the Package Insert.

9. Summary of Preclinical Studies

9.1. Accuracy Study

An accuracy study was carried out to demonstrate that ID CORE XT can accurately determine polymorphism genotypes, the predicted allele genotypes and the predicted antigen phenotypes. ID CORE XT results were compared with different comparator methods, as follows:

- Polymorphism genotypes: ID CORE XT results were compared to bi-directional sequencing (BDS).
- Predicted allele genotypes: ID CORE XT results were compared to BDS-predicted genotypes.
- Predicted phenotypes:
    - ID CORE XT results were compared to phenotype results generated using FDA licensed serology reagents, when available (for the antigens C, E, c, e, K, k, Jka, Jkb, Fya, Fyb, M, N, S and s)
    - ID CORE XT results were compared to BDS-predicted phenotypes for antigens with no FDA licensed serology reagents.

Acceptance criteria: For polymorphism genotypes/phenotypes represented by 299 or more samples, the lower bound of the one-sided 95% CI for percent of correct calls had to be greater than 99%. For polymorphism genotypes/phenotypes represented by fewer than 299 samples, the acceptance criterion was 100% correct calls for all samples tested.

The study included a total of 1676 well-characterized samples (1672 valid test samples). The initial analysis identified a total of 17 discordant samples (25 results). Discordant results from 8 samples (11 results) were resolved in favor of the ID CORE XT assay. Discordant results from 7 samples (10 results) were resolved against the ID CORE XT assay, and relevant limitation statements are included in the package insert. Two discordant samples (4 results) were not resolved after investigation due to unconfirmed serology data and an invalid test, respectively. These two samples were excluded from further analysis. Table 3 provides more details about these discordant samples and discrepancy resolution.

Table 3: Summary of discordant results from internal accuracy study and their investigation

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 favor	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 favor	BDS sample mix-up
Duffy	2	BDS	*FY*:c.125G>A	N/A	In favor	BDS sample mix-up
MNS	3	BDS	*GYP*. Hybrid	Mia (MNS7)	In favor	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.

Conclusion: After discrepancy resolution and sample exclusion, the acceptance criteria for polymorphism genotypes were met except for RHCE:c.307T (lower bound of the one sided 95% CI: 98.5%). One of the 317 samples with RHCE:c.307T was discordant due to a non-previously reported rare allele at RHCE:c.203. This type of discrepancy is described as a limitation of the test in the Package Insert.

9.2. Determination of Limit of Detection

The Limit of Detection (LoD) for the ID CORE XT assay was determined in accordance with (b) (4). The study was performed with the aim of determining the lowest and highest concentrations of DNA at which 95% of the sample replicates resulted in correct sample results.

The ID CORE XT assay lower LoD was determined to be (b) (4). The lowest DNA quantity providing 100% correct results was 10 ng/µl. The upper LoD of the ID CORE XT assay was above 200 ng/µL. Subsequently, a DNA concentration of 20 ng/µl was tested in other validation studies, such as Contamination Titration Study, Cross Contamination Study, Interfering Substances Study, Guard Banding Study and Control Surrogate QC Material Study.

Conclusion: The recommended DNA concentration for ID CORE XT is 20 ng/µl.

9.3. Blood Sample Stability

A study was carried out to determine the stability of whole blood samples stored at 2-8 ºC prior to DNA extraction. Genomic DNA was extracted from EDTA anti-coagulated whole blood samples that had been stored at 2-8 °C for (b) (4) after sample collection and tested with the ID CORE XT. All the polymorphism genotypes and predicted phenotypes of the (b) (4) individual extractions per sample at all points were concordant with the results obtained by BDS.

Conclusion: Whole blood samples collected in EDTA can be stored at 2-8 ºC for (b) (4) prior to DNA extraction.

9.4. DNA Sample Stability

A study was conducted to determine the stability of extracted genomic DNA samples stored between -15 ºC and -20 ºC and tested with the ID CORE XT at the following time-points: (b) (4) after DNA extraction. Correct polymorphism genotype and predicted phenotype results were obtained for every sample at all time-points.

Conclusion: Extracted DNA samples can be stored between -15 and -25 ºC for (b) (4).

9.5. DNA Extraction

Studies were performed to test the possible impact of different DNA extraction procedures and different EDTA anticoagulants on the performance of the ID CORE XT assay: manual procedure using the QIAamp DSP DNA Blood Mini kit, (b) (4) method, and QIAsymphony DSP DNA Mini Kit. All the polymorphism genotypes and predicted phenotypes results were 100% concordant with those expected for every sample in the studies.

Conclusion: The three DNA extraction methods, as well as the three different types of EDTA anticoagulant in blood collection tubes, are acceptable for use with the ID CORE XT.

9.6. Cross-contamination

A study was designed and performed to demonstrate that the test does not exhibit detectable cross-contamination and/or carryover between samples during processing. All genomic DNA samples and replicates provided correct polymorphism genotype and predicted phenotype results, regardless of the sample placement on the hybridization plates. Additionally, all negative control samples met the acceptance criteria to be considered as valid.

Conclusion: The study demonstrated that the ID CORE XT assay does not exhibit detectable cross-contamination and/or carryover between DNA samples or between DNA samples and negative controls during processing.

9.7. Effect of Potentially Interfering Substances

The effect of different potential interfering substances on the performance of ID CORE XT was evaluated using whole blood samples from random donors that were spiked with high levels of following potential interfering substances:

- Endogenous substances: hemoglobin, bilirubin, triglyceride-rich lipoprotein, protein.
- Exogenous substances: ampicillin, acetaminophen, ibuprofen, aspirin, warfarin, heparin, atorvastatin and diphenhydramine.
- Microorganisms and RNA - High concentration of RNA ((b) (4)) in the DNA samples, or the presence of 103-104 CFU/ ml of microorganisms described in (b) (4) .

Conclusion: The performance of ID CORE XT was not affected by the tested potential interfering substances.

9.8. Guard Banding and Surrogate QC Material Studies

Guard Banding and Surrogate QC material studies were performed to evaluate the impact of variations at key assay procedures on the ID CORE XT performance, and to determine whether ID CORE CONTROL was as sensitive as genomic DNA samples to protocol variations. Based on a risk analysis, a total of (b) (4) test case groups were evaluated covering the most critical steps of the four main processes of the ID CORE XT assay (Amplification, Hybridization, Labeling and Data Acquisition).

Conclusion: The results of these studies demonstrate that when the assay is run within the suggested parameters, the test performs as expected. The ID CORE CONTROL is a suitable surrogate QC material to control for errors during ID CORE XT processing.

As a cautionary measure, the end users are required to save and verify the use of the correct PCR program settings in each thermal cycler run. A warning included in the Package Insert states that ID CORE CONTROL samples may not indicate procedural errors if less than 24 µl of ID CORE XT Beads Master Mix are pipetted.

9.9. Intermediate Precision Study

An Intermediate Precision Study was carried out to evaluate the repeatability of ID CORE XT

among operators (2x), instruments (2x), reagent lots (3x), and days (6x). Each operator tested 10 samples in (b) (4) with each ID CORE XT lot on each day run. Each operator used the three reagent lots, alternating between two Luminex instruments, on six non-consecutive days, over a total period of at least (b) (4) days. The observed percent of correct calls was 100% for polymorphism genotypes, predicted allele genotypes and predicted phenotypes for all tests.

Conclusion: ID CORE XT assay demonstrated acceptable intermediate precision across operators, instruments, reagents lots, and days.

9.10. Reagent Stability Studies

9.10.1. Shelf-life Stability Study

The shelf-life stability of the ID CORE XT was evaluated using four reagent lots that were stored at 2- 8 ºC for 3, 6, 9, 10, 12, 13, 15 and (b) (4) months after manufacturing. One of the lot was manufactured with aged raw materials. At each time point of evaluation, (b) (4) replicates of the Sample Panel were tested per reagent lot. For all time points tested for each reagent lot, all polymorphism genotype and predicted phenotype results were 100% concordant with expected results.

Conclusion: ID CORE XT is stable for at least 15 months when stored at 2−8 ºC.

(b) (4)

9.10.3. Shipping Stability Studies

Three shipping stability studies were carried to ensure that ID CORE XT kits will remain stable after their distribution process to the final customers. Actual shipping of the product was performed to a simulated end customer in the US using the actual shipping containers. Upon arrival the ID CORE XT kits were stored at 2−8 °C. The product performance was evaluated upon arrival, and at months (b) (4) from the date of manufacture. The temperatures reached during the shipping process met the acceptance criteria, and correct genotype and phenotype results were obtained in every case. In addition, a simulated shipping stability study and a stress stability study were conducted in which unfavorable shipping conditions were simulated, including extreme temperatures ((b) (4)) and mechanical testing ((b) (4)). Kits were returned to normal storage conditions (2-8 ºC) after the stress cycle. At every testing time point and for all samples and replicates, 100% correct polymorphism genotypes and phenotypes were obtained.

Conclusion: The ID CORE XT kits remain stable up to at least (b) (4) months after having been exposed to either actual shipping conditions to the US, or to a simulation of unfavorable shipping conditions.

9.11. Cross-hybridization Study

Known samples were tested to evaluate any potential non-specific hybridization between ID CORE XT probes and PCR amplification products. The results obtained in this study showed that, in every case, when a given set of probes was not included in the PCR reaction, a "Low Signal" or "Absent" result was obtained for that specific probe set, indicating that no cross-hybridization occurred.

Conclusion: Sequence specificity and absence of cross-reactivity for the primers and probes used by ID CORE XT were confirmed.

10. Summary of Clinical Studies

10.1. Method Comparison Study

This study was conducted in three U.S. clinical sites: American Red Cross (ARC), LifeShare Blood Centers (LBC), and BloodWorks Northwest (BNW). The results from the ID CORE XT assays performed by operators in the clinical laboratories were compared to the following comparator methods:

- Bi-directional sequencing results for genotypes;
- FDA-licensed serology tests for Lub and Jkb;
- Bi-directional sequencing for hrB, hrS, Mia, Yta, Ytb and CW (not detected by a comparable FDA-approved genotyping kit predicating blood group antigen phenotypes and without commercially available FDA-licensed serology);
- FDA-approved genotyping kit for all other phenotypes

Discrepancy resolutions were performed at New York Blood Center (NYBC) following pre-determined discrepancy resolution decision trees.

The study initially tested 1026 samples collected from blood donors. After this initial study, 100 additional samples from patients were included. There was one invalid ID CORE XT run out of the total 35 runs because of negative control failure that was attributed to an error with the Luminex instrument maintenance. The repetition of this run provided a valid run result. All the valid runs provided valid test results for every sample tested.

Acceptance criteria: For polymorphism genotypes/phenotypes represented by 299 or more samples, the lower bound of the one-sided 95% CI for the percent of correct calls should be greater than 99%. For polymorphism genotypes/phenotypes represented by fewer than 299 samples, there must be 100% correct calls for all samples tested.

After comparing ID CORE XT results to the reference methods described above, there were a total of 14 samples with discordant results. Discordant results from 11 samples (12 results) were resolved in favor of ID CORE XT assay. Discordant results from one sample (two results) were resolved against ID CORE XT assay. Two samples (8 results) were excluded from further analysis due to sample mix-up or operator error with serology testing.

Table 4: Summary of discordant results from the method comparison study and their investigation

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 favor	BDS sample mix-up
Rh	1	BDS	*RHCE*:c.676G>C	N/A	In favor	Presence of variant *RHCE:* c.801+219G>T
Rh	1	BDS	*RHCE*:c.307T>C	N/A	In favor	BDS sample mix-up
Kell	2	BDS	*KEL*:c.578T>C	N/A	In favor	BDS sample mix-up
Duffy	1	BDS	*FY*:c.1-67T>C	N/A	In favor	BDS sample contamination
Duffy	1	BDS	*FY*:c.1-67T>C	N/A	In favor	BDS sample contamination
MNS			*GYPA*:c.[59C>T]	N/A	In favor	
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 favor	Presence of *RHCE*Ce[733G]* allele
Rh	1	Comparable product	N/A	C (RH2)	In favor	Presence of *RHD*DIIIa* allele
Rh	1	Comparable product	N/A	VS (RH20)	In favor	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 favor	Presence of variant *LU*:c.1615A>G

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

Conclusion: After discrepancy resolution and sample exclusion, all polymorphism genotypes and antigens met the acceptance criteria.

10.2. External Reproducibility Study

An External Reproducibility study was performed to demonstrate the reproducibility of the ID CORE XT assay across sites/instruments (3x), operators (2 per site), and days (6 per operator). Within a given run, (b) (4) replicates of 10 different DNA samples were tested.

Initially, a total of 36 individual ID CORE XT runs were performed (3 sites x 2 operators per site x 6 runs per operator). However, one "Invalid Run" result was obtained (PCR product may not have been added to the hybridization plate), which required the repetition of the entire run,

providing an overall 97.22% of valid runs. After repetition, all the (b) (4) (36 runs x 10 samples x (b) (4) replicates per sample) individual analysis from Valid Runs provided 100% concordant results with the expected polymorphism genotypes, predicted allele genotypes, and predicted phenotypes for all samples and replicates for all factors (Day/Run, Instrument/Site, Operator).

Conclusion: ID CORE XT assay demonstrates acceptable reproducibility across operator, instrument/site, and day/run.

11. Inspections

11.1. Manufacturing Facilities Review/Inspection

FDA conducted an inspection at the following facility:

Progenika Biopharma,S.A.
Ibaizabal Bidea Edifici, 504 Parque Teconologido De
Bizkaia, 48160
Spain
FEI: 3006413195

The inspection did not reveal any significant deviations from the Quality System Regulation (QSR) or adverse practices. No FDA-483, Inspectional Observations was issued.

11.2 Bioresearch Monitoring (BIMO) Inspections

CBER Bioresearch Monitoring (BIMO) conducted inspections at the three testing sites in the United States. These inspections did not reveal significant problems that would impact the data submitted in this PMA.

12. PANEL RECOMMENDATIONS

This device was not presented to a device panel.

13. CBER DECISION

The available data provide reasonable insurance that ID CORE XT is safe and effective for its intended use and support approval of the ID CORE XT.