Harmony® IVD Kit

P/N 08011281001

Instructions for Use

FOR *IN VITRO* DIAGNOSTIC USE

CE 2797

Concerto Imager IVD

P/N 09101721001

Included with sales part numbers listed below:

P/N 09337423001 (230V) and P/N 09337393001 (120V)

CE IVD

PROFESSIONAL USE ONLY

Roche Sequencing Solutions, Inc
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INTENDED USE

The Harmony® prenatal test is a qualitative assay intended to assess the probability of fetal chromosome 13, 18, and 21 trisomy; the probability of fetal 22q11.2 deletion*, the probability of aneuploidy of fetal sex chromosomes; and to determine fetal sex by analysis of plasma-derived cell-free DNA (cfDNA) from a pregnant woman.

The results are intended for prenatal screening and are not intended to be the sole basis for diagnosis. Harmony test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmatory fetal diagnostic testing, parental evaluation, clinical genetic evaluation, and counseling, as appropriate. Reporting of results is intended to be performed by a clinical laboratory director and provided directly to the ordering healthcare provider for consideration with other clinical assessments.

The Trisomy 21 test option is CE marked under Annex II, List B. All other test options (i.e., Trisomy 13, 18, fetal 22q11.2 deletion*, and fetal sex chromosome aneuploidies) are CE marked under Annex III.

Note: Because the fetal sex determination test option does not have a medical purpose, it does not meet the definition of an IVD device and therefore is not a CE marked product.

*The 22q11.2 test option is not available for those customers in Germany and the UK using the AMB protocol

SUMMARY OF THE TEST

Plasma specimens obtained from pregnant women contain variable quantities of fetal cfDNA.[1-3] The amount of fetal cfDNA in most cases is sufficient to enable assessment of probability for fetal chromosomal aneuploidy.[4]

The Harmony prenatal test (Harmony test, Harmony) utilizes a targeted amplification technology termed DANSR[5] (Digital ANalysis of Selected Regions) and an analysis algorithm termed FORTE[6] to analyze selected regions of the genome in cfDNA from pregnant women to aid in the detection of fetal chromosomal conditions. The Harmony test was initially commercialized as a laboratory developed test in 2012, with several peer reviewed publications demonstrating Harmony's clinical performance.[3,7-16] A large prospective study in a general screening population of pregnant women demonstrated superior performance of the Harmony laboratory developed test over first trimester combined screening, a conventional screening approach using serum protein measurements and ultrasound assessment.[13] The clinical performance of the Harmony test described in this Instructions For Use (IFU) has been found to be comparable to that of the Harmony laboratory developed test.[14,15,17]

The Harmony test evaluates cfDNA specimens from pregnant women in order to assess the probability of trisomy of fetal chromosomes 13, 18, and 21; the probability of fetal 22q11.2 deletion; the probability of aneuploidy of fetal sex chromosomes (monosomy X, XXX, XXY, XYY, XXYY); and to determine fetal sex.[15]

PRINCIPLE OF THE PROCEDURE

The Harmony test is intended for use in analysis of cfDNA samples isolated from plasma from pregnant women who meet the following criteria:

- Maternal age ≥ 18 years
- Gestational age ≥ 10 weeks
- Number of fetuses ≤ 2

The Harmony test requires cfDNA that has been isolated using a commercially available cfDNA extraction kit from approximately 4mL of plasma collected using a cell-free DNA collection tube (Roche PN 07785666001 or equivalent). The QiaSymphony SP/AS nucleic acid extraction platform, MagnaPure 24 platform, and MagnaPure 96 platform have been validated for use with the Harmony test on AcfS.[17]

The Harmony test includes the following components: the Harmony IVD Kit (P/N 08011281001) and the Ariosa cell-free DNA System (AcfS) Software (P/N 07831773001), including the FORTE_R.DLL and FORTE algorithm. The

Harmony test is designed to be used with a set of required equipment and AcfS Software, collectively termed the Ariosa cell-free DNA System.

The Harmony test enables execution of 3 sequential processes on sets of 48-96 specimens which includes 48-95 cfDNA specimens from plasma of pregnant women and 1 Assay Performance Control (APC). First, the Harmony IVD Kit implements the DANSR assay process to amplify a set of universal polymerase chain reaction (UPCR) products from genomic intervals on chromosomes 1-12, 13, 18, 21, 22, X, and Y in each sample, and to quantify the amount of each UPCR product in each sample by hybridization to a custom oligonucleotide microarray. Next, the FORTE_R.DLL and FORTE algorithm evaluate the microarray fluorescence intensity data to compute the probability of trisomy of fetal chromosomes 13, 18, and 21; the probability of fetal 22q11.2 deletion; the probability of aneuploidy of fetal sex chromosomes (Monosomy X, XXX, XXY, XYY, XXYY); and to determine fetal sex in each specimen.

The Sex Chromosome Aneuploidy Panel and the 22q11.2 test option have only been validated in singleton pregnancies.

DANSR Assay Targeted Amplification

A targeted amplification process termed DANSR[5,14,15] is used to simultaneously amplify from each of the DNA specimens UPCR products corresponding to approximately 7000 genomic intervals across the chromosomes of interest.
The specimens are first purified to eliminate potential impurities in the cfDNA. The purified cfDNA specimens are then denatured to expose 3'OH ends for biotinylation, and Terminal deoxy Transferase (TdT) is used to biotinylate the 3'OH ends of the single stranded cfDNAs.

Trios of DANSR assay oligonucleotides targeting specific genomic loci are then annealed to the biotinylated cfDNA. The cfDNAs are then captured onto streptavidin (SA)-coated magnetic beads and washed, and the annealed DANSR assay oligonucleotide trios are ligated. The left and right ends of all DANSR assays contain 5' and 3' extensions, respectively, corresponding to universal PCR (UPCR) primer binding sites. The ligated DANSR assay products are eluted from the SA-beads and used to inoculate UPCR reactions. Thermal cycling of the UPCR reactions is performed in a post-PCR laboratory and yields DANSR assay UPCR products.

The DANSR assay targeted amplification process is performed using an Roche configured Library Robot (Roche P/N 07759371001) to execute specimen and reagent manipulation and specimen and reagent container barcode scanning. Reaction incubations are performed using a Bio-Rad C1000 thermal cycler.

Microarray-Based Quantification of DANSR Assay UPCR Products

The DANSR assay UPCR products are quantified using identical copies of a custom oligonucleotide microarray configured on an Array Of Pegs (AOP) to enable simultaneous processing.[14,15] Each microarray contains 16 redundant copies of approximately 7,000 unique features, where each feature consists of a specific oligonucleotide sequence.

The DANSR assay UPCR products undergo an additional round of amplification and are then purified to create high-concentration DANSR assay UPCR products. The DANSR assay UPCR products are digested resulting in a set of DANSR assay microarray targets that contain a unique sequence complimentary to one of the sequences on the microarray. The DANSR assay microarray targets are hybridized to microarrays on a single AOP. The AOP is washed to remove excess target, labeled using a fluorescently conjugated oligonucleotide, and washed again. The AOP is imaged using a Concerto Imager IVD to quantify the fluorescence signal corresponding to each feature for each sample on the AOP. The Concerto Imager IVD generates a file for each array containing the fluorescence intensity values for each feature on the array.

The microarray-based DANSR assay quantification process is performed by an Roche configured Detection Robot (Roche P/N 07759363001) to execute specimen, reagent, and array manipulation, as well as specimen container, reagent container, and array barcode scanning. Reaction incubations are performed using a Bio-Rad C1000 thermal cycler. Array hybridization is performed using a Binder incubator KB53. Array imaging is performed using a Concerto Imager IVD.

FORTE Algorithm Analysis

The FORTE[6,14,15] algorithm is used to analyze the fluorescence intensity data from the 48-96 unique biological samples. The FORTE algorithm aggregates data from the features for each DANSR assay to obtain a robust median intensity for each DANSR assay in each sample. The FORTE algorithm then normalizes the relative intensities of the DANSR assays to eliminate systematic sample, locus, and allele biases. The FORTE algorithm next evaluates the relative intensities of DANSR assays corresponding to the two alleles of each polymorphic locus to estimate the allele frequency of the polymorphic locus in each sample. The FORTE algorithm then identifies loci that are informative for estimating fetal fraction in each sample (i.e., loci where the maternal genotype is homozygous for one allele, and the fetus has inherited a different allele), and uses the allele frequencies of these informative loci to estimate the fraction of fetal DNA in each sample. The FORTE algorithm next evaluates the relative intensities of DANSR assays corresponding to non-polymorphic loci to estimate the relative concentration of each of chromosomes 13, 18, 21, X and Y, as well as the 22q11.2 chromosomal region*. The FORTE algorithm next assesses the probability of trisomy of chromosomes 13, 18 and 21, the probability of fetal 22q11.2 deletion*, and the probability of fetal sex chromosome aneuploidy (monosomy X, XXX, XXY, XYY, XXYY), by computing the relative likelihood of obtaining the observed chromosome concentration and fetal fraction data from an aneuploid sample versus from a euploid sample. The FORTE algorithm adjusts the raw probability scores for each sample for the prior probability associated with the maternal age and gestational age of the sample. The FORTE algorithm then caps these adjusted probability scores at 0.01% and 99%. For trisomy and sex chromosome aneuploidy, the FORTE algorithm classifies capped probability scores <1% as low probability, and probability scores of ≥1% as high probability. For 22q11.2*, the FORTE algorithm classifies probability scores of <1% as no evidence of a deletion observed, and probability scores of ≥1% as high probability of a deletion. In addition, the FORTE algorithm also evaluates the relative intensities of chromosome Y loci to determine fetal sex. For more information on the AcfS Software, see IFU-1258 AcfS Software (including the FORTE algorithm).

*The 22q11.2 test option is not available for those customers in Germany and the UK using the AMB protocol

MATERIALS PROVIDED

The Harmony test consists of two components: the Harmony IVD Kit (Roche P/N 08011281001) and the Ariosa cell-free DNA System (AcfS) Software (Roche P/N 07831773001), including the FORTE_R.DLL algorithm. The Harmony IVD Kit includes reagents and materials to enable analysis of 8 sets of runs with a batch size of 48-95 plasma-derived cfDNA samples from pregnant women plus one assay performance control (APC). Table 1 lists the components of the Harmony IVD Kit, and Tables 2 through 7 list the contents of each component kit. For materials needed for AMB Protocol (for Germany and UK customers), see IFU-1290 FM1.

NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

Table 1: Harmony IVD Kit Components*

Name	Maximum Number of Tests	Roche P/N	Shipping
DANSR LIBRARY BOX 1	8 X 96	07955707001	Ambient
DANSR LIBRARY BOX 2	8 X 96	07955669001	Dry Ice
DANSR DETECTION BOX 1	8 X 96	07955693001	Ambient
Array 96s384 BOX 2	8 X 96	07955618001	Ambient
DANSR DETECTION BOX 3	8 X 96	07955677001	Ambient
DANSR DETECTION BOX 4	8 X 96	07955642001	Dry Ice

*For materials needed for AMB Protocol (for Germany and UK customers), see IFU-1290 FM1

Table 2: DANSR LIBRARY BOX 1 (P/N 07955707001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
AM1T	Ariosa Magnetospheres 1	250 mL Bottle	150 mL	1	2 to 8°C	08482675001
AM2T	Ariosa Magnetospheres 2	250 mL Bottle	200 mL	1	2 to 8°C	08482683001
AM3T	Ariosa Magnetospheres 3	250 mL Bottle	200 mL	1	2 to 8°C	08482756001
R2B	Resuspend 2 Buffer	250 mL Bottle	175 mL	1	2 to 8°C	08482691001
LEB	Ligate Equilibrate Buffer	500 mL Bottle	300 mL	1	2 to 8°C	08482721001
R4B2	Resuspend 4 Buffer 2	250 mL Bottle	200 mL	1	2 to 8°C	08482748001
#NAP	Nucleic Acid Plate Barcode	Barcode	N/A	8	20 to 25°C	08798800001
#UNA	96-well UNA Plate Barcode	Barcode	N/A	8	20 to 25°C	08798818001
#AM1	AM1 Plate Barcode	Barcode	N/A	8	20 to 25°C	08798826001
#AM2	AM2 Plate Barcode	Barcode	N/A	8	20 to 25°C	08798834001
#AM3	AM3 Plate Barcode	Barcode	N/A	8	20 to 25°C	08798842001
#TCP	Thermal Cycle Plate Barcode	Barcode	N/A	8	20 to 25°C	08798893001
#PPP	Purify & Prepare Plate Barcode	Barcode	N/A	8	20 to 25°C	08798907001
#ETH	Ethanol Barcode	Barcode	N/A	2	20 to 25°C	08798958001
#TD-AM1T	Ariosa Magnetospheres 1 Trough Barcode	Barcode	N/A	16	20 to 25°C	08798869001
#TD-AM2T	Ariosa Magnetospheres 2 Trough Barcode	Barcode	N/A	16	20 to 25°C	08798877001
#TD-AM3T	Ariosa Magnetospheres 3 Trough Barcode	Barcode	N/A	16	20 to 25°C	08798885001
#TD-ETH	Ethanol Trough Barcode	Barcode	N/A	16	20 to 25°C	08798966001
#TD-R2B	Resuspend 4 Buffer 2 Trough Barcode	Barcode	N/A	16	20 to 25°C	8798974001
#TD-LEB	Ligate Equilibrate Buffer Trough Barcode	Barcode	N/A	16	20 to 25°C	08798982001
#TD-LWB	Ligate Wash Buffer Trough Barcode	Barcode	N/A	16	20 to 25°C	08799008001
#TD-R4B2	Resuspend 4 Buffer 2 Trough Barcode	Barcode	N/A	16	20 to 25°C	08799016001

# indicates barcode labels

Table 3: DANSR LIBRARY BOX 2 (P/N 07955669001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
PPPT	Purify and Prepare Plate	50 mL Tube	18 mL	1	-30 to -15°C	08484023001
TCPT	Thermal Cycle Plate	50 mL Tube	36 mL	1	-30 to -15°C	08484015001
BTM	BioTinylate Master Mix	15 mL Tube	1.7 mL	8	-30 to -15°C	08482845001
ADB3*	Anneal DNA Buffer 3*	15 mL Tube	6 mL	8	-30 to -15°C	08482861001
LBB*	Ligate Bead Buffer*	15 mL Tube	5.1 mL	8	-30 to -15°C	08482870001
LIM	Ligate Master Mix	15 mL Tube	6 mL	8	-30 to -15°C	08482888001
LWB	Ligate Wash Buffer	250mL Bottle	200 mL	1	-30 to -15°C	08482896001
APC	Assay Performance Control (Euploid)	10mL Tube	1.9 mL	1	-30 to -15°C	08798788001

*GHS08 Health Hazard Symbol: H360 'May damage fertility or the unborn child' applies to the hazardous material formamide, which is included in ADB3 and LBB buffers. Refer to DANSR Library Box 2 Safety Data Sheet for appropriate formamide-specific hazardous material handling.

Table 4: DANSR DETECTION BOX 1 (P/N 07955693001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
AIB	Array Imaging Buffer	250 mL Bottle	225 mL	1	2 to 8°C	08482772001
PHB	Post Hyb wash Buffer	500 mL Bottle	350 mL	1	2 to 8°C	08482799001
PLB	Post Label wash Buffer	500 mL Bottle	350 mL	1	2 to 8°C	08484392001
R7B2	Resuspend 7 Buffer 2	250 mL Bottle	175 mL	1	2 to 8°C	08482764001

Table 5: Array 96S384 Box 2 (P/N 07955618001) Contents

Component	Name	Vessel	Quantity	Storage	Roche P/N
AOP 96	Array of Pegs	Array	1	2 to 8°C	07955618001

Table 6: DANSR DETECTION BOX 3 (P/N 07955677001) Contents

Component	Name	Vessel	Quantity	Storage	Roche P/N
BT	Blue tray	Plastic	4	20 to 25°C	07871759001
GSK	Gaskets	Rubber	10	20 to 25°C	07871767001
PST	Plate scan tray	Plastic	8	20 to 25°C	08805032001
ST	Stain tray	Plastic	8	20 to 25°C	08805024001
#ETH	Ethanol Barcode	Barcode	2	20 to 25°C	08798958001
#ALM	Array Label Mix Barcode	Barcode	10	20 to 25°C	08798940001
#PHB	Post Hyb Buffer Barcode	Barcode	4	20 to 25°C	08798923001
#PLB	Post Label Buffer Barcode	Barcode	4	20 to 25°C	08798931001
#TD-R7B2	Resuspend 7 Buffer 2 Trough Barcode	Barcode	16	20 to 25°C	08798915001
#TD-ETH	Ethanol Trough Barcode	Barcode	16	20 to 25°C	08798966001
#TD-UPM2	Universal Primer Mix 2 Trough Barcode	Barcode	16	20 to 25°C	08799024001
#TD-HAB	Hyb Anneal Buffer Trough Barcode	Barcode	16	20 to 25°C	08799032001
#TD-AIB	Array Imaging Buffer Trough Barcode	Barcode	16	20 to 25°C	08799105001

# indicates barcode labels

Table 7: DANSR DETECTION BOX 4 (P/N 07955642001) Contents

Component	Name	Vessel	Volume	Quantity	Storage	Roche P/N
ALM	Array Label Mix	50 mL Tube	22 mL	8	-30 to -15°C	08481857001
UPM2	Universal PCR Mix 2	15 mL Tube	13 mL	8	-30 to -15°C	08481849001
HAB	Hyb Anneal Buffer	250 mL Bottle	140 mL	1	-30 to -15°C	08481865001

MATERIALS AND ACCESSORIES REQUIRED

The Harmony test is designed to be used with a set of required equipment and software, collectively termed the Ariosa cell-free DNA System (AcfS). Tables 8 through 10 list the components of the AcfS.

Table 8: AcfS Equipment

Equipment	Source	P/N	Quantity
Concerto Imager IVD	Roche (F. Hoffmann-La Roche, Ltd.)	09101721001 included with 09337423001 (230V) and 09337393001 (120V)	1
Concerto Imager Workstation	Roche (F. Hoffmann-La Roche, Ltd.)	08051844001	1
Library Robot, with Workstation, Monitor and Power Cord	Roche (F. Hoffmann-La Roche, Ltd.)	Library Robot: 07759371001 Workstation: 08464103001 or 09121633001; Monitor: 07871899001; Power Cord: 07759568001	1
Detection Robot, with Workstation, Monitor and Power Cord	Roche (F. Hoffmann-La Roche, Ltd.)	Detection Robot: 07759363001 Workstation: 08464103001 or 09121633001; Monitor: 07871899001; Power Cord: 07759568001	1
AcfS L&D Equipment Installation Bundle	Roche (F. Hoffmann-La Roche, Ltd.)	07759274001	1
AcfS Analysis Server	Roche (F. Hoffmann-La Roche, Ltd.)	07759282001 or 09121641001	1
Binder KB53 Incubator, with Big Bear 1mm throw Plate Shaker	Roche (F. Hoffmann-La Roche, Ltd.)	230V: 07759398001 or 08478007001 120V: 08041652001 or 08478074001	1

Table 9: AcfS Software, including FORTE_R.DLL (P/N 07831773001)

Software	Source
Director	Roche Sequencing Solutions, Inc.
Analysis Service	Roche Sequencing Solutions, Inc
FORTE_R.DLL	Roche Sequencing Solutions, Inc
Re-Analysis Service	Roche Sequencing Solutions, Inc
Report Generator	Roche Sequencing Solutions, Inc

Table 10: AcfS IVD User Guide

Document	Source
AcfS IVD User Guide	Roche Sequencing Solutions, Inc

Table 11: Materials required but not provided

Item	Source	P/N	# per kit
Thermal Cycler C1000	Bio-Rad	185-1197	1 (2 required)
Centrifuge 5810 with microplate rotor*	Eppendorf	5810000068, 53513-872	1 (2 required)
EDTA, disodium, 0.01M*	VWR	BDH7621-1	1 L
Ethanol, 70%*	Teknova	E0030	850 mL
TWEEN 80, 100%*	Sigma	P5188-100ML	100 mL
PCR plate, hard shell, 0.2mL, 96-well	Bio-Rad	HSP-9601	24
Storage plate, square well, 2.2 mL, 96-well	Thermo Fisher	AB-0932	24
Storage plate, 0.8 mL, 96-well^	Thermo Fisher	AB-0765	8
Disposable trough, 100 mL	Tecan	10613048	9
Trough carrier, 100 mL, 3-Position	Tecan	10613020	1 (2 required)
Disposable trough, 320 mL^	VWR	25608-904	3
Trough carrier, 320 mL, 3-Position	Tecan	30116581	1
Adhesive plate seal*	Bio-Rad	MSB-1001	100
DNA LoBind Tube, 1.5mL*	Eppendorf	1130 108.051	8
Pipette, single-channel, L-200XLS^*	Rainin	17014391	1
Pipette tip, 200 µL, filter, sterile*	Rainin	17014963	672

* Or equivalent.

^ For AMB protocol (for Germany and UK customers): 16 storage plates, 0.8 mL, 96-well AB-0765 and 2 disposable trough, 320 mL are needed

SAFETY INFORMATION

Safety Data Sheets (SDSs) for the Harmony IVD Kit can be obtained from https://www.e-labeling.eu/ using the key codes listed in Table 12.

Table 12: SDS Key Codes*

Kit Component	Roche P/N	SDS Key Code
DANSR Library Box 1	07955707001	ARD8000SDS1
DANSR Library Box 2	07955669001	ARD8000SDS2
DANSR Detection Box 1	07955693001	ARD8000SDS3
DANSR Detection Box 3	07955677001	N/A
DANSR Detection Box 4	07955642001	ARD8000SDS4
Array 96s384 Box 2	07955618001	N/A

*For SDS Key Codes for AMB Protocol (for Germany and UK customers), see IFU-1290 FM1

Handle and dispose of Harmony IVD Kit reagents according to the SDSs. Appropriate precautions (including use of personal protective equipment (PPE)) should be used when handling and disposing of Harmony IVD Kit reagents.

Blood and plasma specimens should be considered potentially infectious material. Appropriate precautions (including use of PPE) should be used when handling potentially infectious specimens.

Appropriate precautions (including use of personal protective equipment (PPE)) should be used with automated instrumentation and magnetic equipment. Operate automated instrumentation according to the manufacturer's instructions and the AcfS IVD User Guide.

STORAGE AND HANDLING REQUIREMENTS

Upon receipt of the Harmony IVD Kit, verify the contents of the kit using the MATERIALS PROVIDED tables above. If any Harmony IVD Kit component is missing or damaged, contact your local Roche field applications scientist.

DANSR Library Box 2 and DANSR Detection Box 4 are shipped on dry ice. If there is no residual dry ice in the outer shipping container, or if the outer shipping container has been opened during shipping, contact your local Roche field applications scientist.

Store the Harmony IVD Kit components at recommended temperatures as indicated in the MATERIALS PROVIDED tables above.

Expiration dates can be found on the reagents within the kit. Do not use reagents after the expiration date.

NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

AM1T, AM2T, AM3T, TCPT, and PPPT are each provided in one bulk container, and must be dispensed into eight 96 well plates each (termed AM1, AM2, AM3, TCP, and PPP plates, respectively) prior to use, according to the Reagent Preparation Section of the IFU. Dispensed AM1, AM2, and AM3 plates have the same expiration date of their respective bulk container configurations when stored at 4°C (2 to 8°C). Dispensed TCP and PPP plates have the same expiration date of their respective bulk container configurations when stored at -20°C (-30 to -15°C).

BTM, ADB3, LBB, LIM, ALM, and UPM2 are each provided as eight single use aliquots, where each aliquot contains sufficient material to process one batch of 48-95 samples and 1 APC. Do not reuse residual reagents in single use aliquots. If stored at recommended temperatures and used within 6 hours of removal from storage to 20 to 25°C, these reagents may be used until the expiration date stated on their respective labels.

R2B, LEB, R4B2, LWB, AIB, PHB, PLB, and R7B2 are each provided in one bulk container containing sufficient volumes to enable processing of eight batches of 48-95 samples and 1 APC. If stored at recommended temperatures when not in use, used within 6 hours of removal from storage to 20 to 25°C, and subsequently returned to recommended storage temperatures, these reagents may be used for eight such cycles until the expiration date stated on their respective labels.

HAB is provided in one bulk container containing sufficient volumes to enable processing of eight batches of 48-95 samples and 1 APC. If stored at -20°C (-30 to -15°C) prior to use, used within 6 hours of removal from storage to 20 to 25°C, and subsequently returned to 4°C (2 to 8°C), this reagent may be used for eight cycles until the expiration date stated on its label.

The APC is provided in one bulk container, and should be dispensed into eight individual aliquots each prior to storage in order to avoid multiple freeze-thaw cycles, according to the Reagent Preparation Section of the IFU. Aliquoted APC has the same expiration date of its respective bulk configuration when stored at -20°C (-30 to -15°C).

SPECIMEN COLLECTION, TRANSPORT, STORAGE, AND PREPARATION

1. It is recommended that two (2) tubes of maternal whole blood* are collected by venipuncture using the Roche Cell-Free DNA Collection tube (P/N 07832389001 or equivalent), according to the manufacturer's instructions. Transport and store the whole blood specimen according to the cfDNA-compatible blood collection tube manufacturer's instructions.

2. Separate maternal plasma by centrifuging the whole blood specimen at ambient temperature at 1,600 rcf (g) for 10 to 15 minutes, with minimum acceleration and deceleration. After centrifugation, carefully decap the blood tube and remove the top plasma layer to an appropriate container, taking care to avoid the opaque buffy coat layer which contains the maternal blood cells. Additional centrifugation of the separated plasma may be performed but is not required if the preceding step is done appropriately. Blood specimens collected in the Roche Cell-Free DNA Collection tubes are stable for up to 7 days from draw if stored between 18 and 25°C. Plasma specimens transferred from the blood collection tube are stable for 5 days from transfer if stored between 20 and 25°C and up to 3 years if stored at -30 to -15°C.

3. Isolate cfDNA from approximately 4mL maternal plasma using a commercially available DNA isolation kit or established in-house procedure that has been validated for use in extracting cfDNA from plasma according to the manufacturer's instructions. The QiaSymphony SP/AS nucleic acid extraction platform, MagnaPure 24 platform, and MagnaPure 96 platform have been validated for use with the Harmony test on AcfS.[17] Use the same cfDNA isolation biochemistry on all specimens to be processed together in a batch. Elute the cfDNA specimen to a final volume of 150µL of elution buffer. For optimal AcfS performance, it is recommended to not store DNA following DNA extraction and to proceed immediately to the "Create UNA" task.

*See QC Review and Reporting of Results section for more information on use of the second tube.

INSTRUCTIONS FOR USE

Professional Use Only

Reagent Preparation*

*AM1 and AM2 for Reagent Preparation not available for use in Germany and the UK through November 2023. Please see IFU-1290 FM1 for instructions on Reagent Preparation for AMB Protocol.

1. The Harmony IVD Kit is configured to enable analysis of 8 sets of 48-96 specimens, including 48-95 plasma-derived cfDNA specimens from pregnant women and 1 APC. Reagent preparation tasks 3-7 accomplish dispensing of 5 reagents (AM1T, AM2T, AM3T, TCPT, and PPPT) that are shipped in bulk form into eight 96 well plates formatted for use in Director tasks executed by the Library Robot and the Detection Robot. Reagent preparation task 8 accomplishes dispensing of 1 APC that is shipped in bulk form into eight individual 1.5mL tubes for storage purposes.

2. Label eight 2.2mL 96 deep well plates (Thermo Fisher P/N AB-0932) with the AM1 barcodes provided with the kit. Execute the Director Prepare AM1 task on the Library Robot to dispense bulk Ariosa Magnetospheres 1 reagent (AM1T) into the 8 AM1 plates. The robot dispenses 98µL AM1T into each well of each of the 8 AM1 plates. Upon task completion, seal the AM1 plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at 4°C (2 to 8°C). Dispensed AM1 plates have the same expiration date as bulk AM1T when stored at 4°C (2 to 8°C).

3. Label eight 2.2mL 96 deep well plates (Thermo Fisher P/N AB-0932) with the AM2 barcodes provided with the kit. Execute the Director Prepare AM2 task on the Library Robot to dispense bulk Ariosa Magnetospheres 2 reagent (AM2T) into 8 AM2 plates. The robot dispenses 144µL AM2T into each well of each of the 8 AM2 plates. Upon task completion, seal the AM2 plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at 4°C (2 to 8°C). Dispensed AM2 plates have the same expiration date as bulk AM2T when stored at 4°C (2 to 8°C).

4. Label eight 2.2 mL 96 deep well plates (Thermo Fisher P/N AB-0932) with the AM3 barcodes provided with the kit. Execute the Director Prepare AM3 task on the Library Robot to dispense bulk Ariosa Magnetospheres 3 reagent (AM3T) into the 8 AM3 plates. The robot dispenses 144µL AM3T into each well of each of the 8 AM3 plates. Upon task completion, seal the AM3 plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at 4°C (2 to 8°C). Dispensed AM3 plates have the same expiration date as bulk AM3T when stored at 4°C (2 to 8°C).

5. Label eight 96 well hard shell PCR plates (Bio-Rad P/N HSP-9601) with the TCP barcodes provided with the kit. Execute the Director Prepare TCP task on the Library Robot to dispense bulk Thermal Cycle Plate reagent (TCPT) containing PCR master mix into the 8 TCP plates. The robot dispenses 27µL TCPT into each well of each of the eight TCP plates. Upon task completion, seal the TCP plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at -20°C (-30 to -15°C) . Dispensed TCP plates have the same expiration date as bulk TCPT when stored at -20°C (-30 to -15°C).

6. Label eight 96 well hard shell PCR plates (Bio-Rad P/N HSP-9601) with the PPP barcodes provided with the kit. Execute the Director Prepare PPP task on the Library Robot to dispense bulk Purify and Prepare Plate reagent (PPPT) containing digestion master mix into the 8 PPP plates. The robot dispenses 11µL PPPT into each well of each of the eight PPP plates. Upon task completion, seal the PPP plates with an adhesive plate seal (Bio-Rad P/N MSB-1001), and store the plates at -20°C (-30 to -15°C). Dispensed PPP plates have the same expiration date as bulk PPPT when stored at -20°C (-30 to -15°C).

7. Aliquot the APC into eight labeled 1.5mL tubes (Eppendorf P/N 1130 108.051) with the APC, lot number, and expiration date. Use a Rainin L-200XLS+ pipette (P/N 17014392) and sterile filtered pipette tips (Rainin P/N 17014963) to aliquot 200µL of the APC into its corresponding eight 1.5mL tubes. Upon task completion, cap the 1.5mL tubes, and store the 1.5mL tubes at -20°C (-30 to -15°C). The APC aliquoted into 1.5mL tubes has the same expiration date as the bulk APC when stored at -20°C (-30 to -15°C) and should be used with the same lot of reagents provided in the DANSR Library Box 2 (P/N 07955669001).

DANSR Assay Targeted Amplification*

*Create UNA task using AM1 and AM2 for DANSR Assay Targeted Amplification not available for use in Germany and the UK through November 2023. Please see IFU-1290 FM1 for instructions on the AMB protocol for DANSR Assay Targeted Amplification for AMB Protocol.

1. Obtain the 48-95 patient-derived cfDNA specimens and the APC to be processed together as a batch (also referred to as a "lane") through the Harmony test. Label a 96-well 800µL/well plate (ThermoFisher P/N AB-0765) with a Nucleic Acid Plate (NAP) barcode. If the samples are not already eluted in the 96-well plate via an automated method, use a pipette (Rainin L-200XLS+, P/N 17014392) and sterile filtered pipette tips (Rainin P/N 17014963) to transfer 150µL of each of 48-95 patient-derived cfDNA specimens and 150µL of the APC into separate wells of the NAP plate, being careful to change tips between samples.

2. If running less than 96 samples, load samples in order from top to bottom then left to right, so that columns are filled before rows (fill A1-H1 before A2). See figure below for sample loading order.

3. Note the destination well for each sample including the APC. Seal the NAP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and proceed immediately to the Create UNA task (step 8 below).

4. Create a NAP File (also termed a Specimen Sample Sheet) containing the information regarding the samples in the NAP plate. The required information is listed in table 13.

   NOTE: The NAP Fileneeds to have a minimum of 48 cfDNA unique specimens from plasma of pregnant women denoted as "Specimen". The assay performance control is not considered a unique specimen and should be labeled as "Control".

5. The NAP Fileis a tab-delimited file consisting of a Column Header row, followed by one row per sample for each of the 48-96 samples in the NAP plate (specimen order should match the order of plate loading with row 1 for the sample in well A1, row 2 for well B1, row 3 for well C1, etc.) as demonstrated in the figure below.

6. The barcode of the NAP plate (AD#-xxxxxxxx-NAP) must exactly match the name of the NAP file. For example, the file name for barcoded plate AD12345678-NAP must be AD12345678-NAP.txt. Mismatched barcodes and file names will cause task failures. The file format must be a tab delimited text (.tab or .txt).

	1	2	3	4	5	6	7	8	9	10	11	12
A	**1**	**9**	**17**	**25**	**33**	**41**	**49**	**57**	**65**	**73**	**81**	**89**
B	**2**	**10**	**18**	**26**	**34**	**42**	**50**	**58**	**66**	**74**	**82**	**90**
C	**3**	**11**	**19**	**27**	**35**	**43**	**51**	**59**	**67**	**75**	**83**	**91**
D	**4**	**12**	**20**	**28**	**36**	**44**	**52**	**60**	**68**	**76**	**84**	**92**
E	**5**	**13**	**21**	**29**	**37**	**45**	**53**	**61**	**69**	**77**	**85**	**93**
F	**6**	**14**	**22**	**30**	**38**	**46**	**54**	**62**	**70**	**78**	**86**	**94**
G	**7**	**15**	**23**	**31**	**39**	**47**	**55**	**63**	**71**	**79**	**87**	**95**
H	**8**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**

Table 13: Specimen Sample Sheet Contents

Column Header	Description	Accepted Values
HL_SpecimenID	A unique identifier for the sample.	Alphanumeric string excluding the following characters: <>:"/|\?*
HL_MaternalAge_Years	Maternal age in years at the expected delivery date.	10-99*
HL_GestationalAge_Weeks	Gestational age of the fetus in whole weeks.	10-40
HL_GestationalAge_Days	Any additional days that were not included in GestationalAge_Weeks of the fetus.	0-6
HL_IVFStatus	Indication of egg donor source.	Self nonself no
HL_EggDonorAge_Years	If applicable, age of the mother providing the donor egg at the time of egg donation.	Empty or 12-76
HL_TestId^	The test ordered for the sample.	Harmony Harmony + SCAP Harmony + MX Harmony + FS Harmony + FS + SCAP Harmony + FS + MX Harmony + 22q Harmony + SCAP + 22q Harmony + MX + 22q Harmony + FS + 22q Harmony + FS + SCAP + 22q Harmony + FS + MX + 22q
HL_NumberOfFetus	The number of fetuses present in the sample.	1 or 2
HL_SampleType	Identifies sample as Specimen or APC.	Specimen or Control

*The accepted range of values for maternal age for the software to run is 10-99 years old. However, the clinical validation of the Harmony prenatal test was only performed on subjects 18 years of age or older, as described in the Principle of the Procedure section of this document.

^22q reporting option is not currently available for customers running AMB protocol

7. Execute the Director Accession Sample Sheet task on the Library Robot to accession the NAP File. Director saves the NAP File to the Analysis Server. Director subsequently appends process data (reagent container barcodes, timestamps, etc.) to this file as the samples proceeds through the Harmony test process. Ultimately, the NAP File will contain the history for the run of the Harmony test process performed on the Robots and Imager and will be used as input to the analysis process of the FORTE algorithm.

   NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

8. Execute the Director Create UNA task on the Library Robot to purify the DNA samples in the NAP plate. In the Create UNA task, the robot transfers 140µL of from each well in the NAP plate to the corresponding well of an AM1 plate containing 98µL of a paramagnetic bead-containing solution (AM1). (For batches of less than 96 samples, the robot will still do the transfer task even without samples in the well.) After incubating the AM1 plate for 5 minutes with mixing at 2000 rpm and 5 minutes without mixing, the robot incubates the AM1 plate for 10 minutes on a neodymium magnet to immobilize the AM1 beads near the bottom of the wells. The robot then transfers the 238µL cfDNA-containing supernatant from each well of the AM1 plate to the corresponding well of an AM2 plate containing 144µL of a second paramagnetic bead-containing solution (AM2). After incubating the AM2 plate for 5 minutes with mixing at 2000 rpm and 5 minutes without mixing, the robot incubates the AM2 plate for 20 minutes on a neodymium magnet to immobilize the AM2 beads near the bottom of the wells. After removing the supernatant from each well of the AM2 plate, the robot washes the AM2 beads with 200µL 70% EtOH (ETH), and then elutes cfDNA from the beads by dispensing 25µL Resuspension 2 Buffer (R2B) to each well. After incubating the AM2 plate for 5 minutes with mixing at 1200 rpm and 5 minutes without mixing, the robot incubates the AM2 plate on a magnet for 2 minutes to immobilize the AM2 beads near the bottom of the wells, and transfers the cfDNA-containing R2B supernatant to a new 96-well hard shell PCR plate (Bio-Rad P/N HSP-9601) labeled with a Unified Nucleic Acid (UNA) barcode.*

9. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate at 95°C for 3 minutes using the C1000 Thermal Cycler program Inc1_UNA to denature the DNA in preparation for biotinylation. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion. Avoid shaking or lifting the plate. After denaturation, the UNA plate can be stored at -20°C (-30 to -15°C) for 3 days.*

10. Execute the Director Biotinylate UNA task on the Library Robot to initiate biotinylation of the cfDNA samples in the UNA plate. In the Biotinylate UNA task, the robot dispenses 8µL of BioTinylation Master mix (BTM) to each well in the UNA plate and then shakes the plate at 1900 rpm for 1 minute.

11. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate at 37°C for 1 hour using the C1000 Thermal Cycler program Inc2_UNA to execute the biotinylation reaction. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion. Avoid shaking or lifting the plate.

12. Execute the Director Anneal UNA task on the Library Robot to anneal the DANSR assay oligonucleotide trios to the DNA samples in the UNA plate. In the Anneal UNA task, the robot dispenses 40µL of Anneal DNA Buffer 3 (ADB3) containing the DANSR assay oligonucleotide trios to each well in the UNA plate and then shakes the plate at 1600 rpm for 1 minute.

13. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate while gradually decreasing the temperature from 70 to 30°C over the course of 2 hours using the C1000 Thermal Cycler program Inc3_UNA to execute the annealing process. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion. Avoid shaking or lifting the plate.

14. Execute the Director Ligate UNA task on the Library Robot to ligate the annealed DANSR assay oligos. In the Ligate UNA task, the robot dispenses 30µL of Ligate Bead Buffer (LBB) containing streptavidin (SA)-coated paramagnetic beads to each well of the UNA plate and then shakes the plate for 1 minute at 1400 rpm. The robot then incubates the UNA plate at 30°C for 30 minutes to allow the biotinylated DNA- DANSR assay oligo complexes to bind to the SA-beads. The robot then incubates the UNA plate for 2 minutes on a neodymium magnet to immobilize the SA-beads near the bottom of the wells. After removing the supernatant from each well of the UNA plate, the robot washes the SA-beads twice with 50µL Ligate Wash Buffer (LWB) by dispensing the buffer to each well, shaking the plate at 1900 rpm for 1 minute, incubating the plate on a neodymium magnet for 2 minutes, removing the supernatant, and repeating this process. The robot then washes the SA-beads twice with 50µL Ligate Equilibrate Buffer (LEB) by dispensing the buffer to each well, shaking the plate at 1900 rpm for 1 minute, incubating the plate on a neodymium magnet for 2 minutes, removing the supernatant, and repeating this process. Next, the robot ligates appropriately annealed DANSR assay oligonucleotide trios by dispensing 37µL of Ligate Master Mix (LIM) to each well of the UNA plate, shaking the plate at 2000 rpm for 10 seconds, incubating the plate at 50°C for 10 minutes, incubating the plate on a neodymium magnet to immobilize the SA-beads near the bottom of the wells, and removing the LIM supernatant from each well. The robot then washes the SA-beads twice with 50µL LEB by dispensing the buffer to each well, shaking the plate at 1900 rpm for 1 minute, incubating the plate on a neodymium magnet for 2 minutes, removing the supernatant, and repeating this process. Finally, the robot dispenses 30µL Resuspend 4 Buffer 2 (R4B2) to each well in the UNA plate and shakes the plate at 1900 rpm for 1 minute.

15. Seal the UNA plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the plate at 95°C for 1 minute using the C1000 Thermal Cycler program Inc4_UNA to denature the ligated DANSR assay trios from the SA-bead immobilized DNA. Remove the UNA plate from the thermal cycler and centrifuge the plate for 1 minute at 250 rcf. Gently remove the seal from the UNA plate in one steady, continuous motion. Avoid shaking or lifting the plate.

16. Execute the Director Inoculate TCP task on the Library Robot to transfer the ligated DANSR assay trios from the UNA plate to a Thermal Cycle Plate (TCP) containing a universal PCR master mix. In the Inoculate TCP task, the robot incubates the UNA on a neodymium magnet for two minutes, transfers 25µL of supernatant containing the eluted, ligated DANSR assay oligonucleotide trios from each well of the UNA plate to the corresponding well of the TCP plate, and shakes the TCP plate at 1600 rpm for 1 minute.

17. Seal the TCP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and transfer the plate to the post-PCR C1000 thermal cycler. Incubate the TCP plate at 95°C for 1 minute, followed by 35 cycles of 95°C for 0.5 minutes and 72°C for 3.5 minutes, followed by 72°C for 5 minutes, followed by 10°C forever, using the C1000 Thermal Cycler program 1_TCP_35 to amplify the ligated DANSR assay trios using universal PCR primers. The TCP can remain at 10°C on the thermal cycler overnight or can be stored at -20°C (-30 to -15°C) for up to 3 days.

Microarray-Based Quantification of DANSR Assay UPCR Products

1. Refer to the AcfS IVD User Guide for detailed instructions on executing the DANSR assay detection process using the Detection Robot.

2. Obtain the TCP plate from the C1000 thermal cycler or from -20°C storage, and allow the plate to equilibrate to room temperature. Centrifuge the TCP at 1000 rcf for one minute. Gently remove the seal from the plate in one steady, continuous motion. Avoid shaking or lifting the plate.

3. Execute the Director Inoculate 2 TCP task on the Detection Robot to add Universal PCR Mix 2 (UPM2) to the TCP. In the Inoculate 2 TCP task, the robot transfers 48µL of UPM2 to each well of the TCP plate and performs 2 pipette mixes.

4. Seal the TCP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the TCP plate at 95°C for 1.5 minutes, followed by 72°C for 8.5 minutes, followed by 10°C forever, using the C1000 Thermal Cycler program 2_TCP_01 to further amplify DANSR assay UPCR products. Proceed directly to the Make PPP task.

5. Obtain the TCP plate from the thermal cycler, and allow the plate to reach room temperature. Centrifuge the TCP at 1000 rcf for one minute. Gently remove the seal from the plate in one steady, continuous motion. Avoid shaking or lifting the plate.

6. Execute the Director Make PPP task on the Detection Robot to purify and concentrate the DANSR assay UPCR products in the TCP plate, to transfer the concentrated UPCR product to a new plate containing PPP reagent, and to digest the DANSR assay UPCR products. In the PPP task, the robot transfers 70µL of each DANSR assay UPCR product in the TCP plate to the corresponding well of an AM3 plate containing 144µL of a paramagnetic sphere-containing solution (AM3). After incubating the AM3 plate for 5 minutes with mixing at 2000 rpm and 5 minutes without mixing, the robot incubates the AM3 plate for 5 minutes on a neodymium magnet to immobilize the AM3 beads near the bottom of the wells. After removing the supernatant from each well of the AM3 plate, the robot washes the AM3 beads with 150µL 70% EtOH (ETH), and then elutes the DANSR assay UPCR products from the beads by dispensing 24µL Resuspension 7 Buffer2 (R7B2) to each well. After incubating the AM3 plate for 1 minute with mixing at 1200 rpm, the robot incubates the AM3 plate on a magnet for 5 minutes to immobilize the AM3 beads near the bottom of the wells, and transfers the DANSR assay UPCR products-containing R7B2 supernatant to a new PPP plate.

7. Seal the PPP plate with an adhesive plate seal (Bio-Rad P/N MSB-1001) and incubate the PPP plate at 37°C for 6 hours, followed by 95°C for 3 minutes, followed by 4°C forever, using the C1000 Thermal Cycler program Inc_PPP to digest the DANSR assay UPCR products. The PPP can remain at 4°C on the thermal cycler overnight or can be stored at -20°C (-30 to -15°C) for up to 3 days.

8. Obtain the PPP plate from the thermal cycler or from -20°C storage, and allow the plate to reach room temperature. Centrifuge the PPP at 1000 rcf for one minute. Gently remove the seal from the plate in one steady, continuous motion. Avoid shaking or lifting the plate.

9. Execute the Director Consolidate 96-AOP task on the Detection Robot to hybridize the digested DANSR assay UPCR products to a new Array of Pegs (AOP). In the Consolidate 96-AOP task, the robot transfers 11µL of Hyb Anneal Buffer (HAB) to each well of the PPP and shakes the PPP at 2100 rpm for 1 minute. The robot then transfers 30µL of liquid from each well of the PPP to the corresponding well of a new Stain Tray (ST). The robot then couples a new AOP to the ST, creating a AOP-ST array hybridization assembly.

10. Remove the AOP-ST array hybridization assembly from the Detection Robot and use 4 array clips to secure the array to the stain tray. Incubate the AOP-ST array hybridization assembly at 700 rpm at 70°C for 30 minutes, followed by incubation at a gradually decreasing temperature from 70°C to 35°C over the course of 2 hours in a Binder KB53 oven. After incubation is complete, retrieve the AOP-ST assembly from the Binder KB53 oven, and remove the array clips. Carefully break the seal between the AOP and the ST using a screwdriver.

11. Execute the Director Label AOP task on the Detection Robot to label the array using a fluorescently labeled oligonucleotide probe. In the Label AOP task, the robot washes the AOP in 35mL Post-Hyb Wash Buffer (PHB). The robot then transfers the AOP to a reagent tray containing 22mL Array Label Mix (ALM), and incubates the ALM tray-AOP assembly at 37°C for 2 hours in a Tecan Monitored Incubation Option (MIO) incubator. The robot then dispenses 175µL Array Imaging Buffer (AIB) into each well of a Plate Scan Tray (PST), washes the AOP in 35mL Post Label Buffer (PLB), transfers the AOP to the PST, and shakes the AOP-PST assembly at 500 rpm for 5 minutes. After the task is complete, return the AIB, PHB, and PLB reagents to 2°C to 8°C storage within six hours.

12. Execute the Director Load Array Imaging System (AIS) task on the Concerto Imager IVD to log the imaging of the AOP. Enter the barcodes of the Concerto Imager IVD, the AOP, and the PST into Director software, and select Save.

13. Use the Concerto Imaging System software to image the AOP. Refer to the AcfS IVD User Guide for detailed instructions on imaging an AOP. Launch the Concerto Imaging System software from the Windows Start Menu. Once the application has launched, press Start to open the Imager drawer. Taking care not to spill AIB, place the AOP-PST assembly into the Imager drawer with the barcode facing to the rear of the instrument. Verify the AOP-PST assembly is seated securely on the drawer tabs and select Load in the Imager application to retract the drawer. The Imager scans the AOP barcode. Select Start in the Imager application to initiate the imaging process.

14. Imaging takes about 1 minute per array. Imaging starts with the top left array. All arrays on the AOP are imaged. Four images are taken of each array, and the Concerto Imaging System software combines the four images from each array into a single DAT file. The software thus generates 96 DAT files per AOP, one DAT file per array. The software analyzes these 96 image files to create 96 corresponding CEL files containing intensity values for each feature. The software saves these 96 DAT and CEL files on the Concerto Imager IVD computer. Once imaging is complete, select Unload in the Imager application to open the Imager drawer. After removing the AOP-PST assembly, select Load to retract the drawer. It is recommended to remove the AOP-PST assembly upon completion of scanning. Leaving a scanned plate inside the imager for a prolonged period may result in reagent precipitation potentially causing damage to the imager.

FORTE Algorithmic Analysis

1. Execute the Director Finish Array Imaging System (AIS) task on the Concerto Imager Workstation to initiate analysis by the FORTE algorithm. In the Finish AIS task, Director copies the 96 DAT and CEL files from the AOP to the Analysis Server. The Analysis Service then instructs the FORTE_R.DLL application to perform the analysis by the FORTE algorithm using the CEL files and the corresponding NAP File.

2. The FORTE algorithm computes capped adjusted trisomy probability scores and probability classifications for T13, T18, and T21; capped sex chromosome aneuploidy probability scores and classification; 22q11.2 deletion classification, and fetal sex for each of the samples and APC in the run.

QC Review and Reporting of Results

1. Execute the Director Create Reports task to review run QC, sample QC, and probability results for the samples and APC. Select the run to review based upon its NAP barcode. Once a run is selected, the Create Reports task populates the interface with the corresponding results.

2. The FORTE algorithm computes a set of 4 run ("Lane") QC metrics for each run of 48-96 samples (i.e. a "lane", defined as a batch of 48-95 individual samples and one APC control), and 6 sample QC metrics for each sample. The FORTE algorithm determines QC pass/fail status for each run and sample using pre-established acceptance criteria. The QC metrics and their acceptance criteria are presented in Tables 14 and 15 below.

Table 14: Run QC Metrics

Run QC Metric	Description	Minimum Criteria	Maximum Criteria
LaneMedianSignal	Median signal across the array.	200	10000
LaneFracTestSamplesPassed	Fraction of test samples that passed sample QC metrics.	0.49	1.0
LaneSignalToNoise	A measure of signal and variance across the entire array.	0.9	1
LaneNoise	A measure of the amount of variance observed across the array.	0.9	1

Table 15: Sample QC Metrics

Sample QC Metric	Description	Minimum Criteria	Maximum Criteria
ArrayQuality	An indication of the overall quality of the array.	0.9	1
Signal	The signal in each sample.	200	10000
FetalFraction	An estimation of the fetal fraction.	0.04	0.6
SampleIntegrity	An indication of the overall quality of the sample.	0.9	1
Noise	A measure of the variance observed within the sample.	0.9	1
SignalToNoise	A measure of the relationship between the signal and the variance within the sample.	0.9	1

3. If any lane QC metric fails, the “ReportRedraw” check box is selected for all of the samples within the lane. This queues the samples for a redraw report to be created. If a sample passes all lane QC metrics and fails any sample QC metric, the “ReportRedraw” check box is selected for the sample. This queues the sample for a redraw report to be created. If a sample passes all lane QC metrics and passes all sample quality metrics, the report type “ReportData” check box is selected. This queues the sample for a standard report to be created.

4. The second tube should only be run when the first tube does not yield a Harmony test result for the probability of trisomy 21, 18 and 13. However, if the first tube fails the QC metric for FetalFraction, request a redraw rather than run the second tube (as the likelihood of failing the FetalFraction QC metric again is high).[18]

5. Do not run the second tube or request a redraw when the first tube does not yield a Harmony test result for the probability of sex chromosome aneuploidy or fetal sex (but does yield a Harmony test result for the probability of trisomy 21, 18 and 13).

6. The Harmony IVD Kit provides an APC to be included in each run. APC is derived from genomic DNA isolated from buffy coats of one female and one male human subject (see demographic data in Table 18). The expected values for the APC are presented in Table 17 (male sex and low probability for trisomy). If the APC passes sample QC and does not yield expected results, select ReportRedraw for all samples. Otherwise, proceed to report results for the48-95 specimens. See Table 16 below for further clarification on use of the APC:

Table 16: Use of the Control

	Sample QC Metric	Aneuploidy Result	Outcome
If APC	**Passes** all 6 sample QC metrics and…	is **low** probability for trisomy,	then, Report on samples within the batch
	Passes all 6 sample QC metrics and…	is **high** probability for one or more trisomy,	then, **do not** report on samples in the batch
	Fails one or more of the 6 sample QC metrics and…	is no result	then, **Lab Director to review and decide** if to report on samples in the batch

Table 17: Expected APC Results

Control DNA	T13	T18	T21	Sex
APC	Low	Low	Low	Male

Table 18: APC Demographic Data

Control DNA	Maternal Age (Years)	Gestational Age (Weeks)	Gestational Age (Days)	IVF Status	Egg Donor Age (Years)	Number of Fetus	Sample Type
APC	30	15	0	Non-Self	30	1	Control

Note: Putting in the incorrect number of fetuses or the incorrect IVF status for the APC will increase the likelihood of QC failure of the APC.

When the individual sample QC metrics and batch (lane) QC metrics are successfully met, they provide necessary and sufficient evidence that the test is performing appropriately. The APC control well of material may fail QC at a low level due to random experimental variances that are not indicative of any reduction in quality of the batch of samples. Therefore, if the control APC well fails a QC metric and gives a no result but the remainder of the batch passes the batch QC metrics, the quality of any sample in that batch that passes all the individual QC metrics is appropriate to report at the discretion of the Laboratory Director.

7. Create a Specimen Data File (SDF) containing information regarding the samples in the original NAP plate. TheSpecimen Data File is a tab-delimited file consisting of a Column Header row, followed by one row per sample for each of the 48-96 samples in the NAP plate, where each row contains information described in Table 19. The file format must be a tab delimited text (.tab or .txt). All column headers are required. However, not all of the fields are required to be filled and can be left blank if the information is not available. Name the file with the NAP plate barcode followed by SDF (e.g., AD12345678-NAP-SDF.txt). TheSpecimen Data File is for patient samples only. Do not include “Control” sample types in the Specimen Data File.

Table 19 : SpecimenData File Contents

Column Name in File	Description	Required
HL_SpecimenID	Specimen identifier, must be the same as the identifier used in the NAP file	Yes
HL_PatientName	Patient name (First and Last)	Yes
HL_DateOfBirth	Patient’s date of birth	No
HL_MRN	Patient’s medical record number	No
HL_OtherId	Patient’s alternate record number	No
HL_CollectionDate	Date sample was collected from patient	No
HL_ReceivedDate	Date sample was received at testing laboratory	No
HL_AccountNumber	Account number for clinic sending sample	Yes
HL_ClinicName	Name of clinic sending sample	Yes
HL_ReferringClinician	Name of referring clinician	Yes
HL_ReferringClinicianFax	Fax number of referring clinician	No
HL_OtherClinician	Name of other clinician for patient	No
HL_OtherClinicianFax	Fax number of other clinician for patient	No
HL_Locale	Preferred report language	No

8. Reports can be generated in 16 different languages. The language a report is generated in is determined by the contents of the HL_Locale column for each sample. Table 20 lists locale code options that may be entered in the HL_Locale field to generate a report in a specific language.
Note: If no value is provided for HL_Locale, the report language defaults to en-US.

Table 20: Locale Codes

Locale Code	Language Reported
en-US	English – United States
en-GB	English – Great Britain
es-ES	Traditional Spanish – Spain
de-DE	German – Germany
pt-PT	Portuguese – Portugal
pt-BR	Portuguese – Brazil
fr-FR	French – France
it-IT	Italian
tr-TR	Turkish
cs-CZ	Czech
pl-PL	Polish
zh-CN	Simplified Chinese (PRC)
nl-NL	Dutch – Netherlands
ja-JP	Japanese
zh-TW	Traditional Chinese – Taiwan
ru-RU	Russian

9. Select the Browse button to the right of the Specimen Data File field. Select SpecimenData File window, select the corresponding Specimen Data File. Director integrates the patient data with its corresponding sample data. The Specimen Data File Report Summary window displays the number of samples that Director was able to integrate with its matching patient data.

10. If a sample has ReportData checked, a report is generated containing capped probability scores and classifications for each of T21, T18, and T13. If ordered, a 22q11.2 classification is reported. If ordered, a sex chromosome aneuploidy (monosomy X, XXX, XXY, XYY, XXYY) or monosomy X probability score and classification is also reported. If ordered, a fetal sex is also reported. If a sample has RedrawReport checked, a report is generated indicating that the specimen failed QC and advising that a new sample may be submitted. If a sample has ReportCancel checked, a report is generated indicating that the specimen failed QC.

PROCEDURAL PRECAUTIONS

1. As with any test procedure, good laboratory technique is essential to the proper performance of this assay.

2. Due to the high analytical sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

3. Reliable results are dependent on appropriate specimen collection, transport, storage, and processing. Follow the procedures in this IFU.

4. Good laboratory practices and careful adherence to the procedures specified in this IFU are necessary to avoid contamination of reagents.

5. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences.

6. It is recommended that reagents from the same kit be used together.

7. The Harmony test requires use of 48-96 unique biological samples per batch (including controls). Therefore, using fewer than 48 unique samples (not including controls) could affect test performance

8. Processing more than one tube from the same biological sample in the same batch may negatively affect test performance.

9. Reassessing an entire lane is not recommended and may negatively affect test performance. It is only recommended to reassess specific samples that require correction of demographic information, or the addition/subtraction of optional tests such as SCAP.

   NOTE: It is necessary for the entire plate to be filled with reagents regardless of number of samples in the batch. Therefore, the same amount of reagent is necessary for batch sizes of 48-96 samples.

10. Ensure demographic information is input correctly in order to generate accurate results. Changing this information for a second run could lead to discrepant results.

11. It is possible to obtain valid trisomy results with inconclusive SCAP results and/or Fetal Sex results. In such cases, testing should not be repeated.

LIMITATIONS

1. The Harmony test is not intended to be used in analysis of cfDNA from pregnancies with more than two fetuses, induced or spontaneous twin demise, mosaicism, partial chromosome aneuploidy, translocations, maternal aneuploidy, maternal transplant, or maternal malignancy.[19]

2. The Harmony test is not intended to be used for standalone diagnostic purposes.

3. The Harmony test is intended for use in analysis of cfDNA samples isolated from plasma from pregnant women of ≥ 18 years of age, of ≥ 10 weeks' gestation, and with ≤ 2 fetuses.

4. The Harmony test has been validated for use on specimens collected using the Roche Cell-Free DNA Collection Tube (PN 07785666001 or equivalent).

5. Use of this product must be limited to personnel trained in the techniques described in this IFU. Training is provided by authorized personnel.

6. The Harmony test is validated for use with cfDNA isolated from ≥ 2mL of plasma per specimen. Use of cfDNA isolated from ≥ 4mL of plasma per specimen is recommended.

7. The Harmony test requires at least 4% fetal cfDNA in order to provide a result. cfDNA samples containing excessive amounts of maternal-derived DNA, cfDNA or genomic DNA, may affect the performance of the test.

8. Certain factors, such as mode of conception (i.e., in vitro fertilization), lower gestational age, higher maternal weight and twin pregnancy may be associated with lower fetal fraction and, as a result, higher no-call rates.[2,21-24]

9. Sex chromosome aneuploidies have only been validated in singleton pregnancies.

10. 22q11.2 deletion has only been validated in singleton pregnancies

PERFORMANCE CHARACTERISTICS

Autosomal Trisomy Clinical Performance

The Harmony test was used to assess the probability of trisomy 21, 18, and 13 in a set of blinded banked samples from 791 singleton, twin, natural and IVF pregnancies previously collected in prospective and observational studies.[15] The 791 pregnancies included 150 trisomic pregnancies and 641 euploid pregnancies, and were collected from centers in Sweden, United Kingdom, and the United States. All subjects either had diagnostic testing (amniocentesis and/or chorionic villi sampling) with fetal chromosomal evaluation by karyotype, fluorescent in-situ hybridization (FISH), or quantitative fluorescent polymerase chain reaction (QF-PCR), or were followed to birth, where evaluation for fetal aneuploidies was performed using newborn examination by a healthcare provider with any suspected aneuploidies at birth confirmed with karyotyping. Results from the Harmony test were compared to clinical genetic testing outcomes to assess Harmony test clinical performance in classifying the probability of autosomal trisomy 21, 18, and 13.

All 641 euploid pregnancies were classified correctly as low probability for all three trisomies (specificity 100%, 95% CI: 99.4 – 100%). Of the 108 trisomy 21 cases, 107 were classified correctly as high probability for trisomy 21, with one trisomy 21 case classified as low probability (sensitivity 99.1%, 95% CI: 94.9 – 99.9%). Of the 30 trisomy 18 cases which produced a probability score, 29 were classified correctly as high probability for trisomy 18. One sample was excluded as direct testing of fetal material (disomic) conflicted with karyotyping (trisomic) with initial sensitivity observed as 96.7%, then resolved to 100% (sensitivity 100%, 95% CI: 88.7 – 100%). All 12 trisomy 13 cases were correctly classified as high probability for trisomy 13 (sensitivity 100%, 95% CI: 75.8 – 100%). Note that given statistical sample size and biological limitations, not all aneuploid fetuses will be classified as high probability and some euploid fetuses will be classified as high probability.

The Harmony test was used in two studies specific to twins.[23,24] Both of these clinical studies included euploid and aneuploid samples that were analyzed using the Harmony test with both microarray and sequencing quantitation of the DANSR assays. One study included 9 twin pregnancies affected with aneuploidy and 206 twin pregnancies confirmed to be euploid.[23] The Harmony test identified all 6 cases of trisomy 21 and 2 of the 3 with trisomy 18. Each of the 206 euploid twin pregnancies was correctly classified. The second study included 13 twin pregnancies affected with aneuploidy and 404 twin pregnancies confirmed to be euploid.[24] The Harmony test correctly classified 8 of 8 cases of trisomy 21. There were 5 samples affected with either trisomy 18 or trisomy 13, and 3 were correctly identified. One euploid sample was reported as high probability for aneuploidy and 403/404 euploid twin pregnancies were reported correctly.

Sex Chromosome Aneuploidy Clinical Performance

The Harmony test was used to assess the probability of fetal sex chromosome aneuploidy (monosomy X, XXX, XXY, XYY, XXYY) in 742 of the 791 specimens described above.[16] Results from the Harmony test were compared to clinical genetic testing outcomes to assess Harmony test clinical performance in classifying the probability of sex chromosome aneuploidy.

Of the 727 euploid pregnancies, 725 were classified correctly as low probability for all sex chromosome aneuploidies (specificity 99.7%, 95% CI: 99.0 – 99.9%). The two discordant high probability results were monosomy X and XXX.[16] All 15 of the sex chromosome aneuploidy cases were classified correctly as high probability for the appropriate aneuploidy (sensitivity 100%, 95% CI: 79.6 – 100%). The cohort of singleton pregnancies included 13 cases of monosomy X, 1 case of 47,XXX and one case of 47,XXY.[16] Note that given statistical sample size and biological limitations, not all fetuses will be classified as high probability and some disomic (XX or XY) fetuses will be classified as high probability.

The Sex Chromosome Aneuploidy Panel has only been validated in singleton pregnancies.

Fetal Sex Clinical Performance

The Harmony test was used to determine fetal sex in 787 of the 791 specimens described above.[16] Results from the Harmony test were compared to clinical genetic testing outcomes to assess Harmony test performance in determining fetal sex. All but one of the 787 pregnancies were classified correctly by the Harmony fetal sex test (accuracy 99.9%, 95% CI: 99.3 – 99.9%).

Note: Because the fetal sex determination test option does not have a medical purpose, it does not meet the definition of an IVD device and therefore is not a CE marked product.

22q11.2 Deletion Analytical Performance*

The Harmony test was used to assess the probability of fetal 22q11.2 deletion within a 3.0 megabase (Mb) region in 122 affected samples and 1614 presumed unaffected samples.[20] Affected samples included simulated pregnancy samples and prospectively collected samples from pregnant women with a fetus with a diagnosis of 22q11.2 deletion confirmed by clinical genetic testing. Simulated pregnancy samples were created by mixing plasma from an adult with 22q11.2 deletion confirmed by clinical genetic testing with plasma from a related or unrelated non-pregnant female adult with no diagnosis of 22q11.2 deletion. Presumed unaffected samples were samples from pregnant women with unknown fetal and maternal 22q11.2 deletion status. “High probability” results in the presumed unaffected group were classified as “false positives”.

Analytical sensitivity and specificity of the Harmony test for fetal 22q11.2 deletion were evaluated. Of the 1614 presumed unaffected samples, 1606 received a Harmony result indicating no evidence of a 22q11.2 deletion (specificity 99.5%, 95% CI: 99.0 – 99.7%). Of the 122 affected samples, 92 received a Harmony result indicating a high probability of 22q11.2 deletion (sensitivity 75.4%, 95% CI: 67.1 – 82.2%).

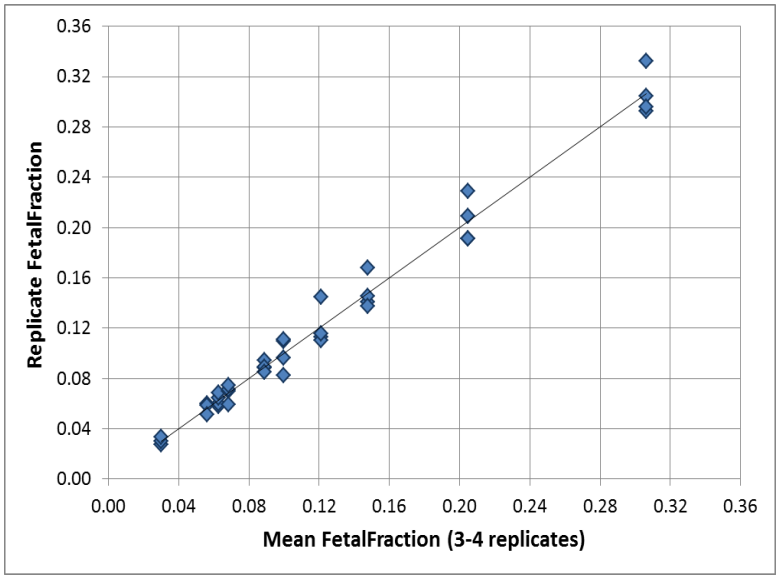
The 22q11.2 test option has only been validated in singleton pregnancies.

*The 22q11.2 test option is not available for those customers in Germany and the UK using the AMB protocol

Fetal Fraction[18]

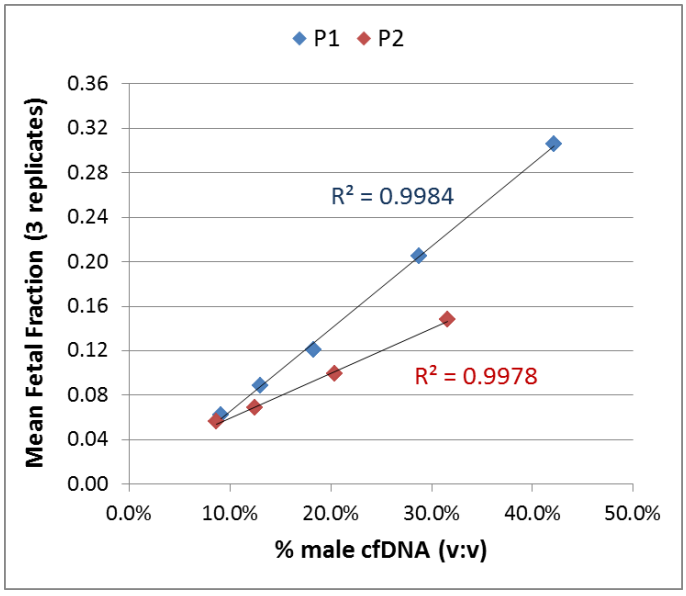
The Harmony test estimates the fraction of the cfDNA sample that originated from the fetus and reports the estimate as the Sample QC Metric FetalFraction. The Harmony test requires samples to have fetal fraction values of 4% or greater in order to provide a result. The analytical performance of the Harmony test fetal fraction metric was assessed by using the Harmony test to evaluate multiple replicates of a set of contrived pregnancy specimens, wherein plasma or cfDNA from plasma from related individuals, one male (contrived fetus) and one female (contrived mother), was mixed in specified proportions. Fetal fraction metrics were evaluated in the resulting data to characterize repeatability, linearity, and limit of detection. Repeatability was assessed by evaluating mixtures with ≥3 replicates at fetal fractions of 3% - 27% and calculating the coefficient of variation (CV) of the Harmony test fetal fraction metric at each of the tested fetal fractions. At all tested fetal fractions, the CV was < 10% (replicate data in Figure 1).

Figure 1: Repeatability of Fetal Fraction



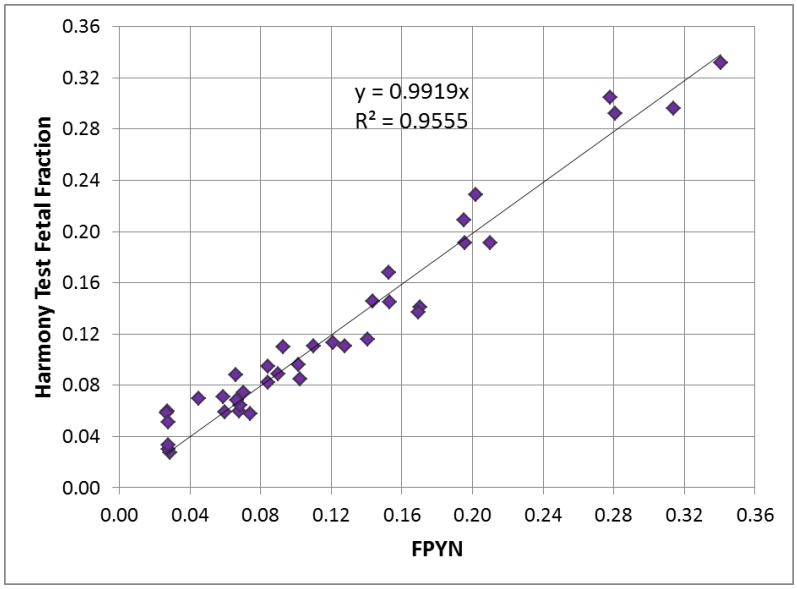
Linearity was evaluated by comparing the Harmony test fetal fraction metric obtained from each mixture with the percentage of total plasma volume in the mixture derived from the male individual. The correlation coefficient (R^2) of the linear regression of the FetalFraction/% male volume comparison was determined for two separate sets of contrived specimens with different subject sources (P1 and P2) with male:female mixtures titrated from 10% male to 45% male. Figure 2 illustrates the strong linear relationship for the two sets of mixtures (P1 and P2) for FetalFraction/% male volume ($R^2 > 0.99$).

Figure 2: Linearity of Fetal Fraction to % Male Volume in Male:Female Mixtures



The ability of the Harmony test to detect minor source cfDNA at fractions from 4% was illustrated in the same set of male:female mixtures by comparing the Harmony test Fetal Fraction metric to FPYN, an independent measure of fetal fraction which uses chromosome Y – specific loci (Figure 3).

Figure 3: Detection of Male cfDNA by the Harmony test



Plasma Input[21]

Over 28,000 blood specimens were collected into the Roche cfD tube or equivalent and were processed through the Harmony prenatal test within 7 days of the blood draw. The Harmony test specimen pass rate and distribution of fetal fraction were evaluated by input plasma volume for volumes from 2mL to 5mL to verify the effectiveness of the Harmony test for this range of specimen input volumes (distribution of specimen input volume is in Table 21).

Table 21 Specimen Input Volume

Plasma input (mL)	# specimens
2.0-2.5	43
2.5-3.0	137
3.0-3.5	561
3.5-4.0	1932
4.0-4.5	6179
4.5-5.0	20077
Total	**28929**

The Effective Pass Rate for a particular plasma input volume was calculated as the ratio of the pass rate for the mL input volume divided by the pass rate of the total dataset. In addition, for the population of specimens at each plasma input volume, the mean and standard deviation of the Harmony test fetal fraction metric was calculated. As illustrated in Figure 4, the Effective Pass Rate was > 90% for all plasma input volumes from 2mL through 5mL; however there is a clear direct relationship of plasma input to Harmony test pass rate. In contrast, the plasma input volume had no effect on the fetal fraction distribution (Figure 5).

Figure 4: Effective Pass Rate by mL of plasma input for the Harmony test

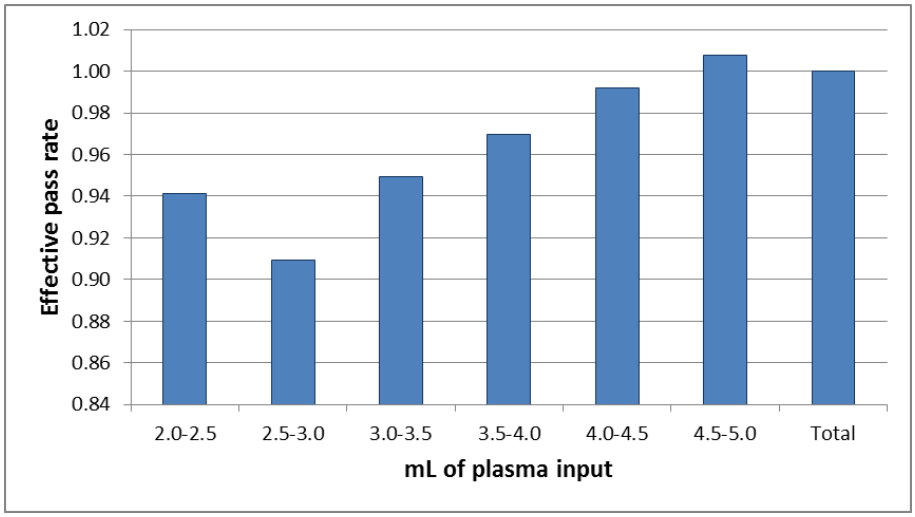
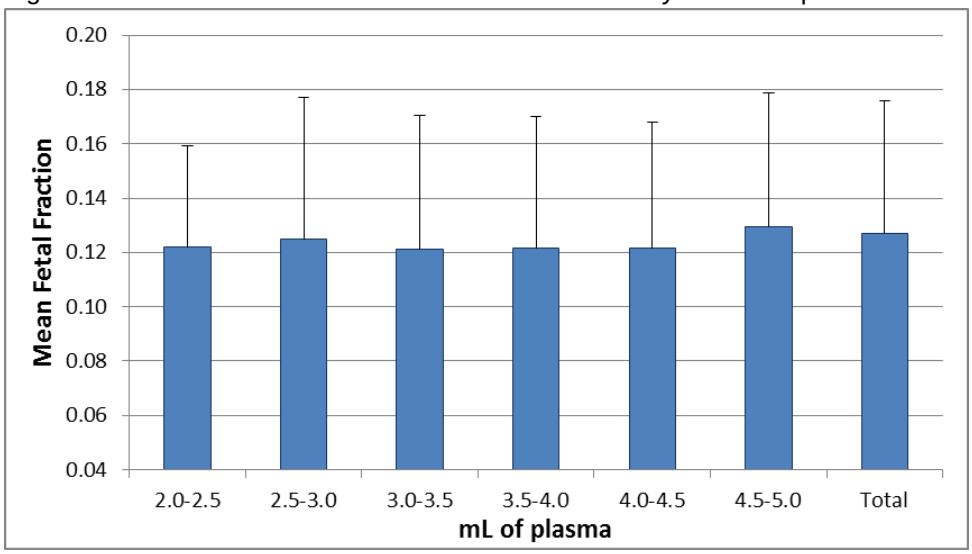


Figure 5: Fetal Fraction Mean and Standard Deviation by Plasma Input Volume



Standard deviation for each series is shown as error bars.

Interfering Substances[21]

To determine if genomic DNA released into the sample from white blood cell lysis could result in fetal cell free DNA fraction failure, replicate samples from 170 subjects were tested. Samples for each individual subject were collected at the same time and included whole blood collected in the Roche cell-free DNA collection tube.

The results from the study showed that the extent of the difference in hemoglobin (Hb) level between samples from the same blood draw do not correlate with any changes in assay pass rate or fetal fraction, even if Hb levels were above 500 mg/dL.

No significant differences were observed in fetal fraction across a range of Hb levels so it is not necessary to reject patient plasma samples due to hemolysis since plasma discoloration caused by hemolysis is not correlated with white blood cell lysis.

The difference in fetal fractions between each sample pair tested was also determined, and the results demonstrated no significant difference in fetal fraction in samples across a range of Hb levels.

TROUBLESHOOTING

Associated Performance Metrics

The six QC metric used to pass or fail a sample are each comprised of several component metrics also called as performance metrics that are incorporated into a single value. In total, fifteen independent measures of quality (performance metrics) are evaluated for each sample. Evaluation of these performance metrics can provide insight to support troubleshooting runs or longitudinal monitoring.These performance metrics are not intented to be used as QC metrics.

Metric Descriptions

Each sample QC metric has associated performance metrics listed. The table below (Table 57) lists the Sample QC metric along with their associated performance metric and the lane QC metrics.

Table 57. Associated Performance metrics

Metric Description	QC Metrics	Associated Performance Metric
Sample Metric	Array Quality	MinStarRobustSigSep
		MaxFracSaturated
		FracGoodFeatures
	Signal	MinNonPolyMedian Signal
	Sample Integrity	FracHom
		FracInfo
		CXXObservedMedianRatio
	FetalFractionQC	FetalFractionQC
	Noise	FPPolyRSE
		MaxNormLogRatioBias
		QuantNormAdjIQR
		AssayAngle
	SignalToNoise	MinNonPolyRobustSigSep
		MinSNR
		MinConfidence

QC metrics are presented in the order in which they should be interpreted; that is, if a sample fails a metric presented earlier in the list, the values of all later metrics are suspect and should not be interpreted to indicate what they are designed to capture. For example, if a sample fails SampleIntegrity, then FetalFractionQC is suspect and should not be viewed as an accurate estimate of the fetal fraction.

ArrayQuality

ArrayQuality provides a single metric evaluating array characteristics that are integral to the quality of the array data, especially with regards to imaging issues.

Possible modes of failure include:

- The wrong array design being used, physical damage to the array, or laboratory processing issues.

- Manufacturing defects on the array may occur at rate of 4% and cause this metric to fail if a large portion of the array is affected by the defect.

• Large amounts of saturating signal on the array (possibly due to contaminating dust), extremely high background noise, or excessive sample signal that interferes with the ability to discriminate different levels of signal between features on the array.

• A large fraction of array features have low signal or there is a high degree of variance across replicate features.

This metric is comprised of three internal metric performance metrics that evaluate the following characteristics and modes of failure:

1. MinStarRobustSigSep

   A measure of whether signal was detected over the features on the array that are unique to the Harmony custom array design. If the wrong array design is used in the Harmony Test process, this metric will fail.

2. MaxFracSaturated

   A measure of the fraction of the array that is overexposed or has saturating signal. If there are large amounts of saturating signal on the array due to contaminating particles on the array, extremely high background noise, or excessive signal from the sample that interfere with the ability to discriminate different levels of signal between features on the array, this metric will fail.

3. FracGoodFeatures

   A measure of the fraction of array features per sample that have non-saturating signal and low variance across replicate array features. If a large fraction of array features have low signal, high variance across replicate features, or no signal at all, this metric will fail.

Signal

Signal measures the strength of the informative signal on the array.

Possible modes of failure include:

- Insufficient cfDNA in the sample.
- Loss of DNA during processing.
- Loss of signal due to insufficient labeling of the sample.
- Insufficient hybridization of the sample to the array.
- Underexposure of the image. Failures can be caused by:
  - Poor cfDNA extraction.
  - Insufficient reagents at certain points of the process.
  - Expired reagents.
  - An expired imaging lamp.

SampleIntegrity

SampleIntegrity provides a single metric evaluating the sample's suitability for analysis based on intrinsic properties of the sample's genetic material, cell-free DNA (cfDNA).

Possible modes of failure include:

- Cross-contamination with another sample.
- The sample has incorrect sample demographic information (e.g., twins instead of a singleton, or nonself egg donor).
- Extremely low or no fetal fraction (for example, a nonpregnant sample).

This metric is comprised of three internal performance metrics that evaluate the following characteristics and modes of failure:

1. FracHom

   A measure of the polymorphic assays based on the genetic knowledge of the relationship of the mother and fetus and in the number of fetuses. Samples which are cross contaminated with another sample, or if the sample has an incorrect demographic (e.g. twins instead of single fetus), or where the genotyping is compromised due to large amounts of array noise will fail this metric. Furthermore, samples which are incompatible with the validated test due to greater than 2 fetuses will fail this metric.

2. FracInfo

   A measure of the polymorphic assays that are used to estimate fetal cfDNA. Samples with extremely low or no fetal fraction will fail this metric, or where the genotyping is compromised due to insufficient resolution of signal in the array.

3. CXXObservedMedianRatio

   A measure of the amount of signal from the X chromosome from a sample. If the major cfDNA source of the sample is male then this metric will fail.

Noise

Noise provides a single metric comprised of four orthogonal internal performance metrics that evaluate the following noise characteristics and modes of failure.

Possible modes of failure include:

- Failures in the reagents or processes during the library preparation process (for example, poor biotinylation of the cell-free DNA, poor annealing of the DANSR assays to the sample, or nonspecific ligation during Ligate UNA).
- Errors in laboratory processes that might result in yield loss.

This metric is comprised of three internal performance metrics that evaluate the following characteristics and modes of failure:

1. FPPolyRSE

   A measure of the variance in the fetal fraction estimate. This metric fails when there is not enough sample diversity in the batch.

2. MaxNormLogRatioBias

   A measure of the variance within a sample for signals from nonpolymorphic assays. This metric will fail if there is insufficient initial cfDNA from the specimen or processing issues during library preparation, especially during hybridization of the sample to the DANSR assays.

3. QuantNormAdjIQR

   A measure of the amount of difference of an individual sample's data from the array data of the batch of samples (lane) in which the sample was processed. This metric will fail if a sample is substantially unlike the other samples on the array. This type of failure may be due to errors in laboratory processing that only affect individual wells, such as a bubble in pipetting that leads to insufficient amount of a reagent dispensed.

AssayAngle

A measure of the inconsistency of a sample's data from the model that a fetus may only have a single trisomic chromosome. This metric may fail if the sample has more than one aneuploidy, unusual genomic conditions, or the genetic material of the sample is substantially unlike the other samples on the array.

SignalToNoise

SignalToNoise provides a single metric comprised of three internal performance metrics that evaluate the following signal to noise characteristics and modes of failure:

1. MinNonPolyRobustSigSep

   A measure of the difference between assay signal on the array from non-specific signal on the array. If the non-specific background signal on the array is too high or the signal from the assays is too low, this metric will fail. Failures in reagents or processes during the detection process, especially during hybridization of the sample to the array will cause this metric to fail.

2. MinSNR

   A measure of the minimum signal to noise observed over all the assays within a sample. This metric considers the allowable amount of variance across chromosomal assays based on the estimated fetal fraction. The allowable variance decreases as fetal fraction decreases. This metric will fail if the fetal fraction is too low or the variance across assays within a sample is too high. This metric often fails when several previous metrics pass but are near the thresholds.

3. MinConfidence

   A measure of the statistical certainty of the observed array data for a sample. If the data from a sample leads to results that are unreliable, this metric will fail. As all aspects of quality influence this metric, this is the metric that fails the largest fraction of samples.

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TABLE OF PART NUMBERS

Table 23: Roche and Ariosa P/N's for Harmony products

Item Description	Roche P/N	Ariosa P/N
Harmony® IVD Kit	08011281001	FGK1002
Ariosa cell-free DNA System (AcfS) Software	07831773001	EQP8006
Detection Robot	07759363001	EQP1101
Harmony® IVD Kit DANSR™ Library Box 1	07955707001	FGK0008
Ariosa Magnetospheres 1 (AM1T)	08482675001	FGS0102
Ariosa Magnetospheres 2 (AM2T)	08482683001	FGS0103
Resuspend 2 Buffer (R2B)	08482691001	FGS0094
Ligate Equilibrate Buffer (LEB)	08482721001	FGS0091
Resuspend 4 Buffer 2 (R4B2)	08482748001	FGS0106
Ariosa Magnetospheres 3 (AM3T)	08482756001	FGS0109
Barcode (AD#-NAP) 8 ea	08798800001	LBL2000
Barcode (AD#-UNA) 8 ea	08798818001	LBL2001
Barcode (AD#-AM1) 8 ea	08798826001	LBL2002
Barcode (AD#-AM2) 8 ea	08798834001	LBL2003
Barcode (AD# -AM3) 8 ea	08798842001	LBL2004
Barcode (AD#-TCP) 8 ea	08798893001	LBL2008
Barcode (AD#-PPP) 8 ea	08798907001	LBL2009
Barcode (AD#-ETH) 2 ea	08798958001	LBL2014
Harmony® IVD Kit DANSR™ Detection Box 1	07955693001	FGK0009
Resuspend 7 Buffer 2 (R7B2)	08482764001	FGS0107
Array Imaging Buffer (AIB)	08482772001	FGS0095
Post Hyb Wash Buffer (PHB)	08482799001	FGS0098
Post Label Wash buffer (PLB)	08484392001	FGS0099
Library Robot	07759371001	EQP1100
Power Cord	07759568001	EQP1023

Item Description	Roche P/N	Ariosa P/N
Array 96S384 Box 2	07955618001	RAW0083
Harmony® IVD Kit DANSR™ Library Box 2	07955669001	FGK0011
Biotinylate Master Mix (BTM)	08482845001	FGS0090
Anneal DNA Buffer 3 (ADB3)	08482861001	FGS0100
Ligate Bead Buffer (LBB)	08482870001	FGS0101
Ligate Master Mix (LIM)	08482888001	FGS0093
Thermal Cycle Plate (TCPT)	08484015001	FGS0105
Purify and Prepare Plate (PPPT)	08484023001	FGS0104
Ligate Wash Buffer (LWB)	08482896001	FGS0092
Assay Performance Control (APC)	08798788001	FGS0126
Harmony® IVD Kit DANSR™ Detection Box 3	07955677001	FGK0012
Stain Tray	08805024001	RAW0084
Scan Tray	08805032001	RAW0085
Blue Tray	07871759001	RAW0071
Gasket	07871767001	RAW0072
Trough Barcode (TD#-PHB) 4 ea	08798923001	LBL2011
Trough Barcode (TD#-PLB) 4 ea	08798931001	LBL2012
Trough Barcode (TD#-ALM) 10 ea	08798940001	LBL2013
Barcode (AD#-ETH) 2 ea	08798958001	LBL2014
Harmony® IVD Kit DANSR™ Detection Box 4	07955642001	FGK0013
Universal PCR Mix 2 (UPM2)	08481849001	FGS0108
Array Label Mix (ALM)	08481857001	FGS0096
Hyb Anneal Buffer (HAB)	08481865001	FGS0097
AcfS Analysis Server	07759282001 or 09121641001	EQP1005

GLOSSARY OF HARMONIZED SYMBOLS

	Temperature limit		Batch code (Lot)
	Use-by-date		Item number
	Do not reuse		Manufacturer
	*In vitro* diagnostic medical device		Consult Instructions For Use
	Contains Sufficient for N tests		Authorized representative in the European Community
	Date of Manufacture		GHS08 Health Hazard: H360 May damage fertility or the unborn child
	Do not dispose of electronic products in the general waste stream (Symbol used for Concerto Only)		ACMA standards compliance label (Symbol used for Concerto Only)
	Global Trade Item Number		Unique Device Identifier

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Harmony IVD Kit

Concerto IVD Imager

TECHNICAL SUPPORT

Please contact your local Roche Representative.

DOCUMENT CHANGE HISTORY

Revision	Valid From	Description of Change Summary
13.0	10-Feb-2021	DCO 6858. Added new AcfS L&D Equipment Installation Bundle to the Materials and Accessories required as the bundle components are required for installation. Added Astersks to prompt UK and Germany customers to see IFU-1290 FM1 for AMB protocol. Added two procedural precautions that already existed but CIR has requested to have documented. Added in an additional limitation that was added to Canada IFU during Health Canada submission. Added two references to that were added from Canada IFU. Updated Specimen Sample Sheet Contents to show that the software is allowing any two digit number for materal age which was added for in AcfS v1.13 to reduce software and user error. Intended opulation remains 18 years of age and older. Added Troubleshooting section to give information on performance metrics. Made a note that the AMB protocol has not been validated for 22q deletion.
14.0	02-Jun-2021	REQ-2020. Corrected the Concerto PN 09337423001 Bundle Sales PN for 230V. Removed China RoHS Compliant (Symbol used for Concerto Only) from GLOSSARY OF HARMONIZED SYMBOLS Section as the product not sold in China. Removed Qarad AR phone number per their internal policy request.
15.0	22-Dec-2021	REQ-4824. Replaced all Training Guide references throughout the document with AcfS User guide (1200000414595). Updated the L&D Workstation and AcfS server PN on Table 10 and 23. Added statement of expected chip defect rate and corrected the rpm and incubation time in the Make PPP task section.
16.0	See EDMS	Removal of DiaDoc reference number for User Guide. Replace references of “Ariosa cell-free DNA system User Guide” with AcfS IVD User Guide.