# ARIOSA® CELL-FREE DNA SYSTEM (ACFS) SOFTWARE

PART NUMBERS:
Roche: 07831773001

## Instructions for Use

FOR IN VITRO DIAGNOSTIC USE

CE
2797

ACFS SOFTWARE V 1.12 AND HIGHER:
FORTE V 4.7.3 AND HIGHER

IVD

**PROFESSIONAL USE ONLY**

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# TABLE OF CONTENTS

**AcfS Software (Including FORTE Algorithm)**
**P/N: 07831773001**

In Vitro Diagnostic Use
IVD

**Instructions for Use**

# INTENDED USE

The AcfS Software, used in conjunction with the Harmony® IVD Kit and a set of required equipment, comprise the Harmony® prenatal test, an automated qualitative assay intended to assess the probabilities of fetal trisomy 21, trisomy 18, trisomy 13, 22q11.2 deletion*, and sex chromosome aneuploidies, 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.

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.[3,7-17]

The Harmony prenatal test (Harmony test, Harmony) consists of the Harmony IVD Kit, a set of required equipment, and the AcfS Software (including the FORTE_R.DLL library and FORTE algorithm). The FORTE algorithm analyzes the imaging data from the Harmony IVD Kit to assess the probability of each of the 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.[15-17] For more information on the Harmony test, see 10100648001 Harmony IVD Kit.

The AcfS Software consists of 5 components: Director, Analysis Service, Re-Analysis Service, Report Generator software, and the FORTE_R.DLL library (and FORTE algorithm). The FORTE algorithm is included in the Analysis Service and Re-analysis Service. The Director software is a graphical user interface and is used to execute tasks on the Library and Detection robots and interacts with Analysis Service software. The Analysis Service captures valuable run-specific data, creates a data backup, and requests FORTE algorithm to begin data analysis. The Re-Analysis Service allows for a set of samples to be re-evauated by FORTE algorithm in the event corrections need to be made to maternal age, gestational age, IVF status, or twin status. The FORTE_R.DLL library, Analysis Service, Re-analysis Service and Report Generator have no direct user interface steps required to perform their tasks. Report Generator is an optional application that interacts with Director and is used to generate a patient report using a predefined template. The FORTE algorithm is used by the Analysis and Reanalysis Services software to generate the probability assessments.

The AcfS Software is installed by a trained Roche Field Service Representative.

## PRINCIPLE OF THE PROCEDURE

The AcfS Software used to evaluate Harmony IVD Kit data is indicated 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 Sex Chromosome Aneuploidy Panel and the 22q11.2 test option have only been validated in singleton pregnancies.

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.

Maternal whole blood is collected by venipuncture and maternal plasma is separated using a cell-free DNA (cfDNA) blood collection tube according to the manufacturer's instructions. CfDNA is isolated and the specimen is processed according to the manufacturer's instructions to produce an array dataset using the Harmony IVD Kit. The AcfS Software is used to retrieve, display and analyze results, including 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 the determination of fetal sex.

# FORTE ALGORITHM ANALYSIS

The FORTE algorithm is used to analyze the fluorescence intensity data from the 48-96 unique biological samples.[6]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 fetal 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.

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

# 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 1 through 3 list the components of the AcfS.

**Table 1 : AcfS Equipment**

| Equipment | Source | P/N | Quantity |
|---|---|---|---|
| Concerto Imager IVD | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 09101721001<br>Included with sales part numbers listed below:<br>09337423001 (230V)<br>and 09337393001 (120V) | 1 |
| Concerto Imager Workstation | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 08051844001 | 1 |

| Equipment | Source | P/N | Quantity |
|---|---|---|---|
| AcfS Library Robot | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 07759371001 | 1 |
| AcfS Library Workstation, Monitor and Power Cord | Roche<br>(F. Hoffmann-La Roche, Ltd.) | Workstation: 08464103001 or 09121633001;<br>Monitor: 07871899001;<br>Power Cord: 07759568001 | 1 |
| AcfS Detection Robot | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 07759363001 | 1 |
| AcfS Detection Workstation, Monitor and Power Cord | Roche<br>(F. Hoffmann-La Roche, Ltd.) | Workstation: 08464103001 or 09121633001;<br>Monitor: 07871899001;<br>Power Cord: 07759568001 | 1 |
| AcfS Analysis Server | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 07759282001 or 09121641001 | 1 |
| Binder KB53 Incubator | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 230V: 07759398001 or 08478007001<br>120V: 08041652001 or 08478074001 | 1 |
| AcfS L&D Equipment Installation Bundle | Roche<br>(F. Hoffmann-La Roche, Ltd.) | 07759274001 | 1 |

**Table 2: 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 3: AcfS User Guide**

| Document | Source | Publication |
|---|---|---|
| AcfS User Guide | Roche Sequencing Solutions, Inc | https://pim-eservices.roche.com/eLD/web/us/en/home |

**Professional Use Only**

These instructions are for personnel who have received training from a qualified Roche representative.

Refer to the *AcfS User Guide* for details regarding operation and maintenance of the AcfS.

# PERFORMANCE AND ANALYTICAL CHARACTERISTICS

The clinical performance characteristics of the AcfS Software and Harmony IVD Kit are supported by studies that evaluate the results of the Harmony prenatal test against other tests or procedures, and/or newborn physical exam performed by a healthcare provider at birth.

## Clinical Performance

### Autosomal Trisomy Clinical Performance

The Harmony test was used to assess the probability of fetal 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, the United Kingdom, and the United States. All subjects either had diagnostic testing (amniocentesis and/or chorionic villi sampling) with fetal chromosomal evaluation by karyotyping, fluorescence 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: 88.3 – 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 performance of the Harmony test with the AMB reagent workflow was assessed based on the concordance with the testing results from the original reagent workflow. 251 clinical samples from

two prospective, observatory multicenter studies were used, which included 34 confirmed aneuploid samples (25 trisomy 21, six trisomy 18, and three trisomy 13) and 217 euploid samples. The concordance analysis was performed on the test results valid for both reagent workflows, with the original workflow as the reference. The overall positive percent agreement between the original and AMB reagent workflows was 97.1% (95% CI: 85.1 – 99.5%) for trisomy aneuploid samples in aggregate (defined as a high probability of trisomy 21, 18, or 13). The overall negative percent agreement for trisomy euploid samples (defined as a low probability of trisomy 21, 18, and 13) between the AMB and original workflow was 100% (95% CI: 98.3 – 100%). The Harmony test with the AMB reagent workflow demonstrated robust testing results, with a clinical performance for detecting common autosomal trisomies (trisomy 21, 18, and 13) equivalent to the original reagent workflow.

Performance of the Harmony test in twin pregnancies has been reported in a study specific to twins.[21] This study included euploid and aneuploid samples that were analyzed using the Harmony test with both microarray and sequencing quantitation of the DANSR assays. Twenty-nine twin pregnancies were affected with aneuploidy. The Harmony test identified 16 of 17 cases of trisomy 21, 9 of 10 cases of trisomy 18, and 1 of 2 cases of trisomy 13. Six false positive results were reported among 968 euploid twin pregnancies (false positive rate 0.62%).

## 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 in the clinical study.[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. 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, one case of 47,XXX and one case of 47,XXY. All 13 monosomy X cases were correctly classified as high probability for monosomy X (sensitivity 100%, 95% CI: 77.2 - 100%) and one false positive was observed (specificity 99.9%, 95% CI: 99.2 - 100%). 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.[16]

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

## 22q11.2 Deletion Clinical Performance

The Harmony prenatal test was used to assess the probability of fetal 22q11.2 deletion within a 3.0 megabase (Mb) region in a set of samples from 735 singleton pregnancies. Samples were obtained through a prospective, multicenter collection of maternal blood from pregnant women who either underwent invasive fetal genetic testing during pregnancy or whose newborns received genetic evaluation and received results for 22q11.2 deletion status and a maternal plasma biobank. Blinded cfDNA analysis by microarray quantitation of DANSR assays targeting a 3.0 Mb region of 22q11.2 was performed and interpreted by the FORTE algorithm. cfDNA test result for 22q11.2 deletion was subsequently compared to that by prenatal or postnatal FISH, microarray and/or comparable technology.[17]

Of 46 maternal plasma samples from pregnancies with a 22q11.2 deletion ranging in size from 1.25 to 3.25 Mb, 32 had a cfDNA result indicating a high probability of deletion (sensitivity 69.6%; 95% CI: 55.2 - 80.9%). In the 689 maternal plasma samples from pregnancies without a 22q11.2 deletion, no false positives were observed (100% specificity, 95% CI: 99.5 - 100%).

The 22q11.w test option 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 in the clinical study.[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.

## Analytical Performance

### Analytical Sensitivity – Limit of Detection (Fetal Fraction)[22]

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%.

### Analytical Specificity – Cross-Reactivity

DANSR oligonucleotide sequences were assessed for similarity to human and non-human genome sequences from the NCBI RefSeq database. An effective percent identity (EPI) statistic was calculated by multiplying the percent identity by the proportion of query sequence covered by the hit. The hits were filtered by EPI using a >90% cutoff. The absence of high quality hits of > 90% EPI amongst bacterial, archaea, viral, fungal, plant, and protist sources indicate that the risk of off-target amplification due to environmental contamination other than primates is low. The assay may be sensitive to human or other primate DNA contamination.

### Accuracy – Precision: Repeatability and Reproducibility

Contrived samples derived from cell line genomic DNA from affected and unaffected patients were used to assess Repeatability and Reproducibility. This study examined the correct call rate of the

Harmony results for all test options compared with known clinical truth and for replicate agreement of results for the following variables: three clinical sites/instruments, six operator teams, three reagent lots, 18 start days, and within-run. In the reproducibility study, the correct call rates were 100% across the 3 sites with 100% agreement across the variables for the T21 panel, the trisomy aneuploid panel (T21, T18, and T13), the euploid panel, and the SCA panel. The 22q11.2 deletion panel demonstrated 91.9% agreement across the variables and correct call rates of 82.9%, 92.9%, and 100% at the three sites. Reproducibility, as evidenced by percent agreement and correct call rate, of all panels exceeded product specifications and demonstrated robust results.

In the lot-to-lot variability study, the correct call rate was 100% for T21, T18, T13, SCA, and euploid samples for all runs, showing no variability in performance across lots, operator teams, or runs. For the 22q11.2 deletion panel, the percent agreement was 97.6%. The first reagent lot had a correct call rate of 100%, the second reagent lot 95.2%, and the third reagent lot 97.5%, again demonstrating minimal variability in performance between lots. Overall, the Harmony prenatal test for all test options demonstrated high sensitivity and specificity with robust reproducibility and repeatability across all the variables of clinical site, equipment, operator, lots, and runs.

## AMB Performance Equivalency

Samples from pregnant women were used to establish equivalency of the performance of the Harmony test with the AMB reagent to the testing results from the original reagent workflow. The samples are from the intended use population and included samples with high probability results for T21, T18, T13, SCAP, and monosomy X.

The trisomy performance equivalency of the Harmony test with the AMB reagent was calculated with a sample set of 2331 pregnant women subjects. The original workflow was used as reference. The 2331 samples included 94 T21, nine T18, and five T13 aneuploid samples with 2224 autosomal euploid samples. For T21, the PPA was 100% (95% CI: 96.1% – 100%). For T18, the PPA was 100% (95% CI: 67.6% – 100%). For T13, the positive present agreement (PPA) was 100% (95% CI: 56.6% – 100%). The NPA was >99.9%, 95% CI 99.8% – 100%. Overall, the agreement for the combined trisomy was >99.9% (95% CI: 99.8% – 100%).

The SCAP performance equivalency of the Harmony test with the AMB reagent was calculated with a sample set of 1062 pregnant women subjects. The original workflow was used as reference. The 1062 samples included four XO, four XXX, five XXY, five XYY and 1043 low risk SCAP samples. PPA was 94.7% (95% CI: 75.4% – 99.1%), and NPA was 99.4% (95% CI: 98.8% – 99.7%). Overall agreement was 99.3% (95% CI: 98.7% – 99.7%).

The fetal sex performance equivalency of the Harmony test with the AMB reagent was calculated with a sample set of 2025 pregnant women subjects. The original workflow was used as reference. The percent agreement for male calls was >99.9% (95% CI: 99.5% – 100%). The percent agreement for female calls was 100% (95% CI: 99.6% – 100%). The overall agreement was >99.9% (95% CI: 99.7% – 100%).

## Clinical Cut-off

Three thousand and twenty-one (3021) samples were used to evaluate the clinical cut-off for T21 and T18, including 80 T21 cases and 38 T18 cases. Nineteen hundred and forty-nine (1949) samples were used to evaluate the clinical cut-off for T13, including ten T13 cases. Five hundred and twenty-three

(523) samples were used to evaluate the clinical cut-off for chromosome Y aneuploidy, including three XYY cases, while four hundred and twenty-nine (429) samples were used to evaluate chromosome X anueploidy, including 32 monosomy X cases and one XXX case, and both studies included six XXY cases. To evaluate the clinical cut-off for 22q11.2 deletion, three hundred and fifty (350) samples were used, including one hundred and eighty-two (182) 22q11.2 deletion cases and one hundred and sixty-eight (168) controls. The risk scores for T21, T18, and T13, sex chromosome aneuploidy, and 22q11.2 deletion for each subject were capped at 99% on the upper end and 0.01% on the lower end. A risk exceeding 1% classified a subject as high risk for T21, T18, or T13, sex chromosome aneuploidy, and 22q11.2 deletion.

# Warnings and Precautions

### Lab practices

- As with any test procedure, good laboratory technique is essential to the proper performance of this assay.
- Due to the high analytical sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination. Good laboratory practices and careful adherence to the procedures specified in this IFU are necessary to avoid contamination of reagents.
- Reliable results are dependent on appropriate specimen collection, transport, storage, and processing. Follow the procedures in this IFU. and 10100648001 Harmony IVD Kit.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[18,19] Only personnel proficient in handling infectious materials and the use of Ariosa cell free DNA System (AcfS) should perform this procedure. If spillage occurs, follow local regulations and guidelines.
- Due to inherent differences between technologies related to steps upfront of the Harmony test i.e. extraction or specimen collection, 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.

### Creating a batch

- 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.

  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.

- Processing more than one tube from the same biological sample in the same batch may negatively affect test performance.
- Review the sample demographic information. Make sure that it is accurately associated with the sample identifier and well position. Changing this information for a second run could lead to discrepant results.
- Confirm that the NAP and Sample Data file contain allowed symbols (see respective sections for details).
- Entering the incorrect number of fetuses or the incorrect IVF status for the APC will increase the likelihood of QC failure of the APC.
- The sample data file does not include the APC.
- When placing your samples in the NAP, do not skip rows, columns, or wells in between samples.
- The barcode of the NAP plate (AD#-xxxxxxxx-NAP) must exactly match the name of the NAP file.

### Assessing a batch

- 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.
- It is possible to obtain valid trisomy results with inconclusive SCAP results. In such cases, testing should not be repeated.
- False positive or false negative result may occur if cross contamination of samples is not adequately controlled during sample handling and processing.

### Executing the assay workflow

- Before starting any robotic step, ensure that the front safety shield of the Library Robot is closed. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument.
- Make sure to check that the BINDER oven is stable at 70°C before use.
- Do not place hands or face near load tray when loading or ejecting plates on the Concerto Imager and do not stare into the laser beam of the Concerto Imager when tray is open.
- Do not open the tray to the Concerto Imager when instrument is running.
- Do not touch the Concerto Imager tray when starting an imaging run or when loading a tray.
- To avoid physical injury while the Concerto Imager is powered up and emitting light. Do not directly look into the light guide.

- The infrared radiation (and ultraviolet radiation) generated by the xenon lamp in the Concerto Imager can cause significant skin burns and eye damage.
- Explosion Hazard. High internal pressure exists in any xenon arc lamp.
- High Voltage Hazard. High ignition voltages exist inside the cabinet and can be lethal.
- Do not attempt to service the Concerto Imager instrument. Removing the case exposes you to laser and electrical shock hazards.
- Use a computer that is connected to the Concerto Imager when entering barcodes for the Load AIS and Finish AIS tasks.

### Contacting your local competent authority

- Inform your local competent authority about any serious incidents which may occur when using this assay.

## LIMITATIONS

- The AcfS Software has not been validated for any diagnostic use or use in analysis of pregnancies with more than two fetuses, induced or spontaneous twin demise, mosaicism, partial chromosome aneuploidy, translocations, maternal aneuploidy, maternal transplant, or maternal malignancy.[20]
- Use of the AcfS Software with any other reagents, consumables, hardware and software has not been validated by Roche Sequencing Solutions, Inc. (RSS). RSS hereby expressly disclaims any and all liability arising out of use of the AcfS Software with any reagents, consumables, hardware or software not approved by Roche Sequencing Solutions, Inc., or for any use other than the intended use specified above.
- The AcfS Software has only been validated for use with reagents, consumables, hardware and software approved in writing by RSS.
- It is each laboratory's responsibility to validate use of the AcfS Software with reagents, consumables, hardware and software not provided by RSS.
- The AcfS Software may not be distributed, reverse engineered, copied, modified or otherwise made available or transferred or disclosed to any third parties not approved by RSS.
- 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]
- The Harmony test is not intended to be used for standalone diagnostic purposes.

- 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.
- The Harmony test has been validated for use on specimens collected using the Roche Cell-Free DNA Collection Tube (PN 07785666001 or equivalent).
- Use of this product must be limited to personnel trained in the techniques described in this IFU. Training is provided by authorized personnel.
- 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.
- 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.
- 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.
- Sex chromosome aneuploidies have only been validated in singleton pregnancies.
- 22q11.2 deletion has only been validated in singleton pregnancies.
- 22q11.2 test option is not available for those customers in Germany and the UK using the AMB protocol.

## SYSTEM REQUIREMENTS

The AcfS Software will execute on 64-bit Windows 10 Enterprise, Windows 2016 Server Standard platforms with a minimum .NET 4.6 environment. The AcfS Software implements LDAP Authentication guarding against unathorized access to the software. Fortigate Firewall provides network security using strict network segmentation. The AcfS Software has been validated when executed on English-US Microsoft Windows environments as specified above.

## TECHNICAL SUPPORT

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

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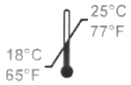
The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed.

## GLOSSARY OF HARMONIZED SYMBOLS

| | | | |
|---|---|---|---|
|   | Temperature limit |   | Batch code (Lot) |
|   | In vitro diagnostic medical device |   | Item number |
|  | Date of Manufacture |  | Manufacturer |
|   | Unique Device Identification |  | Consult Instructions For Use |
|   | Global Trade Item Number |   | Authorized representative in the European Community |
|  | Importer | | |

## DOCUMENT REVISION

| **Document Revision Information** | |
|---|---|
| Doc Rev. 1.0<br>01/2024 | First publishing for Branchburg based on IFU-2323 Rev 4.0.<br>Replaced Santa Clara's footer containing material number (IFU-2323) and Rev (4.0) with Branchburg footer containing material number including revision number (10100761001-01) and Doc Rev. (1.0).<br>Replaced IFU-2322 with 10100648001 in the **Warnings and Precautions** section.<br>Updated **TECHNICAL SUPPORT** section.<br>Please contact your local Roche Representative if you have any questions. |