GenetiSure Dx Postnatal Assay IVDR Registration
Summary of Safety and Performance

Document Information:

Filename	SSP- GenetiSure Dx Postnatal Assay IVDR Registration
Current Owner	Agilent Technologies, Inc.
Project Identifier	GenetiSure Dx Postnatal Assay IVDR Registration CPE
Product Identifier	K1201A
Product Regulatory label	CE-IVD to CE-IVDR

Table of Contents:

1 Purpose of the Document

This Summary of Safety and Performance (SSP) is intended to provide public access to an updated summary of the main aspects of the safety and clinical performance of the device.

The SSP is not intended to replace the Instructions For Use (IFU) as the main document to ensure the safe use of the device, nor is it intended to provide diagnostic or therapeutic suggestions to intended users of the Agilent product GenetiSure Dx Postnatal Assay who are health care professionals.

1.1 Definitions

Table 1. List of Definitions

Acronym/Term	Description
CIV ID	EUDAMED Clinical Investigation Identification number
FSCA	Field Safety Corrective Action
FSN	Field Safety Notification
GSPR	General Safety and Performance Requirements
IFU	Instructions for Use
PER	Performance Evaluation Report
PMPF	Post-Market Performance Follow-Up
PMS	Post-Market Surveillance
PSUR	Periodic Safety Update Report
SSP	Summary of Safety and Performance
SNR	Single Registration Number
UDI-DI	Unique Device Identifier-Device Identifier

1.2 References

Table 2. List of References

Document #	Title
MDCG 2019-9	Summary of safety and clinical performance. A guide for manufacturers and notified bodies
EU IVDR 2017/746	European In Vitro Diagnostic Regulation
ISO 15189:2012	Medical Laboratories – Requirements for Quality and Competence

Document #	Title
EN ISO 14971:2019	Medical devices - Application of risk management to medical devices
IFU K1201-90001	Agilent GenetiSure Dx Postnatal Assay
K1201-90030	Agilent CytoDx Installation & Set Up Guide

2 Scope

This SSP summarizes the main features of the GenetiSure Dx Postnatal Assay and CytoDx Software including identification, intended use, description, risk and warnings related to the device, the summary of the performance evaluation and post market follow up, possible diagnostic alternatives and suggested training for users.

3 General Device Information

Table 3. Overall information of the device

Required Information	Description
Device Trade Name	GenetiSure Dx Postnatal Assay
Manufacturer	Agilent Technologies, Inc. 5301 Stevens Creek Blvd Santa Clara, CA 95051-7295 USA
Manufacturer's Single Registration Number (SRN)	US-MF-000009385
Basic UDI-DI	GenetiSure Dx Postnatal Assay 570057R0403P3011C040000N8 CytoDx Software 570057R0403P3011C040700PB
Medical device nomenclature description	GMDN: 62428
Class of Device	C
Year when the first CE certificate was issued covering the device	*2022*
Authorised Representative	Soren Buch Agilent Technologies Denmark ApS Produktionsvej 42 2600 Glostrup, Denmark soren.buch@agilent.com +45 88305832

Required Information	Description
Authorized Representative SRN	DK-AR-000001443
Notified Body	TÜV SÜD Product Service GmbH Zertifizierstellen Ridlerstraße 65 80339 MÜNCHEN, Germany ps.zert@tuev-sued.de +49 (89) 50084261

4 Intended use of the device

4.1 Intended purpose/Intended use

GenetiSure Dx Postnatal Assay is a qualitative assay intended for the postnatal detection of copy number variations (CNV) and copy- neutral loss of heterozygosity (cnLOH) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. GenetiSure Dx Postnatal Assay is intended for the detection of CNVs and cnLOH associated with developmental delay, intellectual disability, congenital anomalies, or dysmorphic features. Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counseling, as appropriate.

Interpretation of assay results is intended to be performed only by healthcare professionals, board certified in clinical cytogenetics or molecular genetics. The assay is intended to be used on the SureScan Dx Microarray Scanner System and analyzed by CytoDx Software.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing or screening, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

4.2 Indications and intended patient population

GenetiSure Dx Postnatal Assay is a qualitative assay intended for the postnatal detection of copy number variations (CNV) and copy- neutral loss of heterozygosity (cnLOH) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. GenetiSure Dx Postnatal Assay is intended for the detection of CNVs and cnLOH associated with developmental delay, intellectual disability, congenital anomalies, or dysmorphic features.

4.3 Contraindications and/or limitations

Limitations of the Procedures

1) The CytoDx software does not perform aberration detection in the pseudoautosomal regions (PAR) of the Y chromosome. As the PAR sequences are the same on the X and Y chromosomes, in male samples, CytoDx cannot distinguish between a PAR

aberration on the X chromosome and a PAR aberration on the Y chromosome. Consequently, any aberrations identified in a PAR are assigned to the X chromosome. For male patient samples (or any patient sample paired with a male reference sample), probes in Y chromosome PAR regions are included in the Triage View display of the CytoDx software for visualization purposes only. In patient samples that contain an abnormality of the Y chromosome, the abnormalities in the PAR1 and PAR2 regions at the ends of the short and long arms of the Y chromosome are only displayed on the X chromosome. For female patient samples (or any patient sample paired with a female reference sample), probes on the Y chromosome are included in the Triage View display of the CytoDx software for visualization purposes, but the samples are not analyzed for Y chromosome aberrations.

2) The assay should be performed in a licensed clinical laboratory.
3) The assay is for use with gDNA from whole blood specimens only. Blood must be collected in tubes using EDTA as the anticoagulent. It has not been validated for any other specimen type.
4) The assay was validated for use with 500ng of gDNA. Less than 375 ng gDNA may not work.
5) Do not quantify the DNA using absorbance.
6) Mosaicism CN less than 50% may not be reliably detected.
7) The GenetiSure Dx Postnatal Assay cannot identify balanced chromosomal rearrangements, such as translocations or inversions.
8) The smallest regions that the software will report are 20 kb and 5 probes for gains, and 10 kb and 5 probes for losses. At this size range, reproducibility for copy number gains between 20–50 kb is 82%, and reproducibility for copy number losses between 10–50 kb is approximately 76% (refer to the analytical performance in Section 7.2). Performance of the assay has not been assessed for CNVs with size and marker number below these settings for reporting.
9) The smallest regions the software will report for cnLOH are 5 Mb and 100 SNP probes.
10) Links to external databases have not been evaluated or curated by Agilent.
11) GenetiSure Dx Postnatal Assay is limited to personnel trained in this assay.
12) This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing or screening, population screening, or for the detection of, or screening for, acquired or somatic aberrations.
13) Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counseling, as appropriate. Confirmation of microarray results using qPCR, FISH, or another approach is recommended.

14) CNVs that are present in regions with no or low probe coverage may not be detected, including regions in which probes were masked during the CytoDx analysis. The CytoDx software masks probes in regions in which the reference sample contains a CNV and in some regions of hypervariability. The preloaded tracks Agilent Female CNV Reference and Agilent Male CNV Reference contain the masked intervals for the female and male reference samples respectively. You can view these tracks from the Configure Settings > Tracks screen in the CytoDx software.

15) The GenetiSure Dx Postnatal Assay is for use on the SureScan Dx Microarray Scanner.

5 Device description

5.1 Description of the device

Summary and Explanation of the Assay and Cyto Dx Software.

Comparative genomic hybridization (CGH) is a molecular cytogenetic method of screening cells for genomic DNA (gDNA) gains and losses at a sub- chromosomal level. Unlike traditional techniques used to detect CNVs, which rely on the examination of a single target and prior knowledge of the region under investigation, CGH produces a map of gDNA sequence copy number as a function of chromosomal location throughout the entire genome. In traditional CGH, differentially labeled test gDNA and reference gDNA are hybridized simultaneously to chromosome spreads. The hybridization is detected with two different fluorochromes. CNVs are seen as changes in the ratio of the intensities of the two fluorochromes along the target chromosomes [Kallioniemi et al. (1992), Science, 258: 818-21]. The resolution of traditional CGH is limited to alterations of approximately 5- 10 Mb. Array CGH (aCGH) combines the principles of CGH with the use of microarrays [Schena et al. (1995), Science, 270: 467- 470]. This approach overcomes the resolution limitations associated with traditional CGH. Instead of using metaphase chromosomes, glass slides arrayed with small segments of DNA are used [Lucito et al. (2003), Genome Research, 13: 2291- 2305]. On an Agilent aCGH microarray, the DNA segments (known as probes) are created *in situ* directly on a glass slide. Because probes are several orders of magnitude smaller than metaphase chromosomes, the theoretical resolution of aCGH is proportionally higher than that of traditional CGH. The true level of resolution is determined by considering both probe size and the genomic distance between DNA probes. Despite its advantages in CNV detection, aCGH alone cannot detect copy- neutral loss of heterozygosity (cnLOH). In cnLOH, a person's genome includes a chromosome or part of a chromosome that lacks heterozygosity. For example, the person may receive two copies of a chromosome, or part of a chromosome, from one parent and no copies from the other parent due to errors in meiosis I or meiosis II. If the gDNA in a cnLOH interval is imprinted such that the genes in that region are monoallelically active (i.e., only the maternal or paternal allele of the pair is expressed), the resulting phenotype will be abnormal. Standard aCGH microarrays cannot detect cnLOH because they do not contain probes designed to detect single nucleotide polymorphisms (SNPs). To overcome this barrier, Agilent extended their aCGH microarrays to include a set of SNP probes on the same microarray (called a CGH+SNP microarray). Restriction digestion of the sample gDNA allows genotyping of SNPs located in the enzymes' recognition sites.

For each SNP probe, gDNA that has been cut at the restriction site results in a different fluorescent signal than that produced by uncut gDNA. Genotyping of SNPs allows for subsequent detection of cnLOH intervals. Thus, Agilent CGH+SNP microarrays, like those provided in the GenetiSure Dx Postnatal Assay, allow for the simultaneous, high- resolution detection of CNVs and cnLOH intervals.

CytoDx is a workflow based application that is part of the GenetiSureDx Postnatal Assay that helps to analyze, review, and report data. The CytoDx software provides the QC metrics and aberration calls results.

Principle of the Procedure

The assay performed with the GenetiSure Dx Postnatal Assay is an in vitro diagnostic assay for use in a clinical laboratory. The assay is based on aCGH (molecular karyotyping).

Molecular karyotyping is a modified *in situ* hybridization technique that allows detection and mapping of gDNA sequence copy differences between two genomes in a single experiment. In molecular karyotyping analysis, two differentially labeled gDNA (patient sample and reference) are co- hybridized to complementary nucleic acid sequences synthesized *in situ* on a microarray slide.

Locations of copy number variants (CNV) in the gDNA segments of the patient sample genome are revealed by variable fluorescence intensity on the microarray. The CGH+SNP microarray included in the GenetiSure Dx Postnatal Assay uses approximately 107,000 probes for CNV detection. Half of these probes were chosen to selectively hybridize to targeted regions designated by ISCA (the International Standards for Cytogenomic Arrays) to be of clinical interest and the other half were chosen to hybridize to sequences evenly spaced across the whole genome, commonly termed backbone probes.

The probes are distributed on the array, targeting overall 94% of the genome with at least 5 copy number probes per 400 kb, resulting in a median resolution of approximately 150 kb. Regions identified to be clinically relevant are targeted with increased probe density resulting in a median resolution of approximately 25 kb.

The assay compares the patient sample against a sex- matched reference sample. gDNA is extracted from the patient's whole blood and then is fluorescently labeled in parallel with the reference sample using two different fluorescent dyes. The two labeled samples are hybridized to complementary sequences (probes) that are printed on a CGH+SNP microarray.

After hybridization, the microarrays are washed and then scanned. The data from the microarray images are converted to numeric data. The relative abundance of the target sequences is computed based on the relative intensities of the fluorophores in the patient and reference samples hybridized to each of the probe sequences.

The numeric data is then processed using software specifically designed to report CNVs by chromosomal location. The reported CNVs are interpreted by a Board Certified Cytogeneticist, Molecular Geneticist, Molecular Pathologist, or similarly qualified clinician who

has been trained to identify the clinically relevant CNVs, determine clinical significance, and report out these findings. cnLOH in patient samples is also reported to the clinician based upon allele data from the additional 59,647 SNP probes present on the Agilent microarray.

Regions of cnLOH are identified in the software by locating genomic regions with a statistically significant scarcity of heterozygous calls. First, the software's algorithm uses total and allele-specific copy numbers to identify each SNP site as having a homozygous or heterozygous genotype. Then, it uses a binomial probability distribution to report regions that contain an unusually high fraction of homozygous SNPs.

Software to support clinical interpretation of the results of the GenetiSure Dx Postnatal Assay diagnostic test is NOT a component of this device.

5.2 *Reference and comparison to previous generation(s) or variants*

There are no variants of the GenetiSure Dx Postnatal Assay.

5.3 Accessories used in combination with the device

There are no accessories which are intended to be used in combination with the device.

5.4 Other devices and products used in combination with the device

Table 4. Other devices and products intended to be used in combination with the Device

Product Name	Catalog Number	Basic UDI-DI
GenetiSure Dx Labeling Kit	K1201-64100	N/A
GenetiSure Dx Labeling Kit	K1201-64105	570057R0801P3011A040100QM
GenetiSure Dx Labeling Kit Purification Columns	K1201-64110	570057R0801P3011A040100QM
GenetiSure Dx Hybridization Kit	K1201-64200	570057R0801P3011A040100QM
GenetiSure Dx Wash Buffer 1, 4L	K1201-64305	570057R0801P3011A040100QM
GenetiSure Dx Wash Buffer 2, 4L	K1201-64310	570057R0801P3011A040100QM
GenetiSure Dx Cot-1 Human DNA	K1201-64400	570057R0801P3011A040100QM
QIAamp DSP DNA Blood Mini Kit	Qiagen 61104	N/A
SureScan Dx Microarray Scanner	G5761A	570057R0802P3011A040200S3

6 Reference to any harmonised standards and CS applied

Table 5. Other devices and products intended to be used in combination with the Device

No	Standard Developing Organization	Standard Designation Number and Date	Title of Standard
1	EN ISO	14971:2019	Medical devices - Application of risk management to medical devices
2	ISO	ISO 23640:2011	In vitro diagnostic medical devices -- Evaluation of stability of in vitro diagnostic reagents
3	EN ISO	ISO 13485:2016	Quality Management for Medical Device
4	AAMI	ANSI ISO 15223-1:2012	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements
5	AAMI	ANSI IEC 62304:2006	Medical device software - Software life cycle processes
6	AAMI	ANSI IEC 62366-1 Edition 1.0 2015-02	Medical devices - Part 1: Application of usability engineering to medical devices
7	CLSI	EP07-A2 - 05/21/2007	Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
8	CLSI	EP12-A2 - 01/30/2014	User Protocol for Evaluation of QualitativeTest Performance
9	CLSI	EP25-A - 01/15/2013	Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
10	CLSI	MM13-A – 12/01/2005	Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline
11	CLSI	MM21: 1st Edition – Aug 2015	Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications
12	IEEE	IEEE: Std 829-1998	Standard for Software Test Dcoumentation

7 Performance evaluation and post-market clinical follow-up

This section is intended to summarise, in a comprehensive manner, the clinical evaluation results and the clinical data forming the clinical evidence for the confirmation of conformity with relevant general safety and performance requirements, the evaluation of undesirable side-effects and the acceptability of the benefit-risk ratio.

7.1 Summary of performance data related to equivalent device

The Affymentrix CytoScan Dx Assay was used as a predicate device for establishing substantial equivalence to the GenetiSure Dx Postnatal Assay. The equivalent device SSP is not available in Eudamed.

Table 6. Overview of predicate device

	GenetiSure Dx Postnatal Assay (Device)	Affymetrix CytoScan Dx Assay (Predicate) K130313
Indications for Use	GenetiSure Dx Postnatal Assay is a qualitative assay intended for the postnatal detection of copy number variations (CNV) and copy-neutral loss of heterozygosity (cnLOH) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. GenetiSure Dx Postnatal Assay is intended for the detection of CNVs and cnLOH associated with developmental delay, intellectual disability, congenital anomalies, dysmorphic features, or clinical presentation suggestive of a chromosomal syndrome. Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counseling, as appropriate. Interpretation of assay results is intended to be performed only by healthcare professionals, board certified in clinical cytogenetics or molecular genetics. The assay is intended to be used on the SureScan Dx Microarray Scanner System and analyzed by CytoDx Software. This device is not intended to be used for standalone diagnostic purposes, preimplantation or prenatal testing or screening, population screening, or for the	CytoScan® Dx Assay is a qualitative assay intended for the postnatal detection of copy number variations (CNV) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. CytoScan® Dx Assay is intended for the detection of CNVs associated with developmental delay, intellectual disability, congenital anomalies, or dysmorphic features. Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counseling, as appropriate. Interpretation of assay results is intended to be performed only by healthcare professionals, board certified in clinical cytogenetics or molecular genetics. The assay is intended to be used on the GeneChip® System 3000Dx and analyzed by Chromosome Analysis Suite Dx Software (ChAS Dx Software). This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing or screening, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

	GenetiSure Dx Postnatal Assay (Device)	Affymetrix CytoScan Dx Assay (Predicate) K130313
	detection of, or screening for, acquired or somatic genetic aberrations	
Special Conditions	For prescription use	Same.
Sample Type	Peripheral whole blood	Same.
Technology	Microarray for comparative genomic hybridization	Same.
Software	Assay-specific software is used to perform feature extraction, CNV and cnLOH identification and reporting on the microarray images.	Same.
Assay steps	Starts with purified genomic DNA (gDNA) and ends with microarray intensity data.	Same.
Quality Controls	In-process QC checks, external controls and array QC metrics are used to monitor and assess the quality of results.	Same.
What is Reported	The device reports the copy number change (amplification, deletion) and loss of heterozygosity aberrations, and position/location of the aberrant segment across the queried genome.	Same.
Limitations	This device is not intended to be used for standalone diagnostic purposes, pre-implantation of prenatal testing or screening, population screening, or for the detection of or screening for, acquired or somatice genetic aberrations.	Same
Clinical Validation	Compare test results with available diagnosis for sample.	Same

7.2 Summary of analytical and clinical performance

7.2.1 Analytical Performance

(a) Trueness of Measurement

Analytical Accuracy

The analytical accuracy of the GenetiSure Dx Postnatal Assay was investigated by evaluating samples obtained from multiple sources and carrying a wide variety of chromosomal aberrations. Of 626 samples tested in total, 556 eligible samples constituted a comprehensive panel for accuracy evaluation. The diversified sample panel consisted of 451 aberrant genomic DNA (gDNA) samples derived from established commercial cell lines, 76 archived clinical gDNA samples isolated from whole blood specimens of anonymized patients, 5 globally recognized syndrome reference panel gDNA samples, and 24 fresh blood-derived gDNA samples extracted from whole blood of phenotypically normal subjects.

The samples were tested through the GenetiSure Dx Postnatal Assay per standard procedures in a designated Agilent laboratory. The accuracy of detected aberrations, including copy number variations (CNV, reported specifically as amplifications (AMP) or deletions (DEL)) and copy-neutral loss of heterozygosity (cnLOH), was evaluated against corresponding reference data from selected comparator platforms. The extensive variety of aberrations detected across the whole genome (collective coverage 91%) deemed eligible for accuracy evaluation were analyzed through predesigned algorithms that involved various comparators and multiple assessment tiers. Commercially available copy number confirmatory assays based on a validated qPCR technology were employed to assist accuracy assessment of CNV aberrations and resolve discrepancies.

The aberration confirmation rates (% agreement with comparator) calculated using a predefined 65% minimum overlap criterion and a definitive scheme excluding any “indeterminate” calls, were 93.5% for larger CNVs with >20 probes, 92.5% for smaller CNVs with 5-20 probes, and 90.1% for all cnLOH aberrations. As the main performance metrics designated for accuracy evaluation, these results have sufficiently met the predefined acceptance criteria for this study. In addition, refined aberration size binning either by probe number or length in kilobases (kb) was carried out in the accuracy evaluation, which further substantiated the consistently high accuracy performance across a wide aberration size range. Excellent accuracy (25/26, or 96.2% confirmation rate) near the resolution limit for CNV detection was confirmed using the aforementioned qPCR technology on selected small CNVs detected in normal whole blood-derived samples. Alternative assessment criteria for aberration confirmation (agreement) were also explored, with overall concordant outcomes supporting the robustness of the preferred analysis approach. Breakpoint accuracy was evaluated on confirmed aberrations. High levels of breakpoint agreement with comparators were found (91.0% for CNV and 91.4% for cnLOH, respectively)

In summary, these results demonstrate that the GenetiSure Dx Postnatal Assay is highly accurate in detecting chromosomal aberrations across the human genome,

providing a robust analytical basis for its intended use as a contributor to clinical diagnosis.

Table 7. Sample Source and Sex Composition

	Sex				Total	
	Female		Male			
Sample Source	**N**	**Row %**	**N**	**Row %**	**N**	**Column %**
ARUP (Clinical)	32	42%	44	58%	76	14%
BioSpecialty (Normal Blood)	12	50%	12	50%	24	4%
Coriell (Cell Line)	205	45%	246	55%	451	81%
NIBSC (WHO PWS/AS Panel)	2	40%	3	60%	5	1%
All	251	45%	305	55%	556	100%

For all the 556 samples evaluated, 4.1 (Mean) ± 2.1 (SD) CNVs were detected on average per sample (Median=4), of which 2.3 ± 1.4 were small CNVs with 5-20 probes and 1.8 ± 1.6 were large CNVs with >20 probes (Median=2 for both size categories). More descriptive statistics for these 556 samples can be found in (All Samples). These statistics remained almost unchanged if all the aberrant samples were considered together (532 samples from Coriell, ARUP, and NIBSC combined). When considering the 24 normal blood-derived samples only (BioSpecialty), the average number of CNVs per sample was approximately halved: 2.0 (Mean) ± 1.5 (SD), Median=2. The majority of these detected CNVs were small-sized (5-20 probes), with more being DEL than AMP. Large CNVs (>20 probes) detected in normal blood samples were relatively rare (Mean =0.25, Median=0 per sample).

Table 8. Aberration Counts Per Sample

All Samples Included in Evaluation (N= 556)						
Distribution	**AMP**	**DEL**	**CNV 5-20 probes**	**CNV >20 probes**	**CNV-all**	**cnLOH**
Max	27	9	8	28	28	33
90th Percentile	3	4	4	3	6	1
Median	1	2	2	2	4	0
10th Percentile	0	1	1	0	2	0
Min	0	0	0	0	0	0
Mean	1.6	2.5	2.3	1.8	4.1	0.5
Std Dev	1.7	1.6	1.4	1.6	2.1	3.0

Normal Whole Blood Samples Only (N= 24)						
Distribution	**AMP**	**DEL**	**CNV 5-20 probes**	**CNV >20 probes**	**CNV-all**	**cnLOH**
Max	3	5	5	2	5	1
90th Percentile	2	3	4	1	4	0.5
Median	0	1	1.5	0	2	0
10th Percentile	0	0	0	0	0	0
Min	0	0	0	0	0	0
Mean	0.67	1.3	1.8	0.25	2.0	0.08
Std Dev	0.87	1.3	1.4	0.53	1.5	0.28

Table 9. Main Accuracy Assessment

	No. of Aberrations				**Confirmation Rate (95% CI)**		
Category	**Confirmed**	**Indeter-minate**	**Not confirmed**	**Total**	**Scheme a** Excluding indeterminate CNV	**Scheme b** Including indeterminate CNV as "not confirmed"	**Acceptance Criteria**
CNV >20 probes	846	35	59	940	93.5% (91.7%, 94.9%)	90.0% (87.9%, 91.8%)	≥ 90%
CNV 5-20 probes	1082	77	88	1247	92.5% (90.8%, 93.9%)	86.8% (84.8%, 88.5%)	≥ 60%
cnLOH	263	N/A	29	292	90.1% (86.1%, 93.0%)		≥ 80%

The acceptance criteria were met for all of the three main aberration categories. The average confirmation rates calculated based on scheme a were greater than 90% for all categories evaluated (93.5% for large CNVs with >20 probes, 92.5% for small CNVs with 5-20 probes, and 90.1% for all cnLOH). Even if considering the more conservative estimates based on scheme b, the corresponding confirmation rates were still able to meet the pre-defined acceptance criteria.

Table 10. Scheme a: Excluding Indeterminate CNVs

TYPE	Aberration Range (# of Probes)	Sample Size (N)*	# Confirmed	Confirmation Rate (95% CI)**	FPR*** (95% CI)
AMP	5-7	44	39	88.6% (76.0%, 95.0%)	11.4% (5.0%, 24.0%)
	7-10	97	91	93.8% (87.2%, 97.1%)	6.2% (2.9%, 12.8%)
	10-15	188	165	87.8% (82.3%, 91.7%)	12.2% (8.3%, 17.7%)
	15-20	91	83	91.2% (83.6%, 95.5%)	8.8% (4.5%, 16.4%)
	20-50	127	107	84.3% (76.9%, 89.6%)	15.7% (10.4%, 23.1%)
	50-500	78	70	89.7% (81.0%, 94.7%)	10.3% (5.3%, 19.0%)
	500 +	169	166	98.2% (94.9%, 99.4%)	1.8% (0.6%, 5.1%)
	Total	794	721	90.8% (88.6%, 92.6%)	9.2% (7.4%, 11.4%)
DEL	5-7	212	211	99.5% (97.4%, 99.9%)	0.5% (0.1%, 2.6%)
	7-10	187	169	90.4% (85.3%, 93.8%)	9.6% (6.2%, 14.7%)
	10-15	244	229	93.9% (90.1%, 96.2%)	6.1% (3.8%, 9.9%)
	15-20	107	95	88.8% (81.4%, 93.5%)	11.2% (6.5%, 18.6%)
	20-50	122	103	84.4% (77.0%, 89.8%)	15.6% (10.2%, 23.0%)
	50-500	225	220	97.8% (94.9%, 99.0%)	2.2% (1.0%, 5.1%)
	500 +	184	180	97.8% (94.5%, 99.2%)	2.2% (0.8%, 5.5%)
	Total	1281	1207	94.2% (92.8%, 95.4%)	5.8% (4.6%, 7.2%)
All CNVs		2075	1928	92.9% (91.7%, 93.9%)	7.1% (6.1%, 8.3%)
cnLOH	100-200	132	106	80.3% (72.7%, 86.2%)	19.7% (13.8%, 27.3%)
	200-500	102	99	97.1% (91.7%, 99.0%)	2.9% (1.0%, 8.3%)
	500 +	58	58	100.0% (93.8%, 100.0%)	0.0% (0.0%, 6.2%)
	Total	292	263	90.1% (86.1%, 93.0%)	9.9% (7.0%, 13.9%)

* The number of aberrations analyzed in each range bin, excluding indeterminate CNVs

** Confirmation Rate = TP/(TP+FP), equivalent to "# Confirmed / Sample Size (N)". It can also be referred to as "% Agreement" or "Positive Predictive Value (PPV)". 95% CI calculated using the Wilson score method. Applicable to other tables in this report.

*** FPR (False Positive Rate) = Pr (Aberration "Not Confirmed" | Aberration detected by the GenetiSure Dx Postnatal Assay) in this context is "1-Agreement (Confirmation Rate)" rather than the conventional concept of "1-specificity". Applicable to other tables in this report.

Table 11. Scheme b: Including Indeterminate CNVs as "Not Confirmed"

TYPE	Aberration Range (# of Probes)	Sample Size (N)*	# Confirmed	Confirmation Rate (95% CI)	FPR (95% CI)
AMP	5-7	48	39	81.3% (68.1%, 89.8%)	18.7% (10.2%, 31.9%)
	7-10	101	91	90.1% (82.7%, 94.5%)	9.9% (5.5%, 17.3%)
	10-15	197	165	83.8% (78.0%, 88.3%)	16.2% (11.7%, 22.0%)
	15-20	101	83	82.2% (73.6%, 88.4%)	17.8% (11.6%, 26.4%)
	20-50	148	107	72.3% (64.6%, 78.9%)	27.7% (21.1%, 35.4%)
	50-500	82	70	85.4% (76.1%, 91.4%)	14.6% (8.6%, 23.9%)
	500 +	169	166	98.2% (94.9%, 99.4%)	1.8% (0.6%, 5.1%)
	Total	846	721	85.2% (82.7%, 87.5%)	14.8% (12.5%, 17.3%)
DEL	5-7	216	211	97.7% (94.7%, 99.0%)	2.3% (1.0%, 5.3%)
	7-10	202	169	83.7% (77.9%, 88.1%)	16.3% (11.9%, 22.1%)
	10-15	257	229	89.1% (84.7%, 92.4%)	10.9% (7.6%, 15.3%)
	15-20	125	95	76.0% (67.8%, 82.6%)	24.0% (17.4%, 32.2%)
	20-50	130	103	79.2% (71.5%, 85.3%)	20.8% (14.7%, 28.5%)
	50-500	225	220	97.8% (94.9%, 99.0%)	2.2% (1.0%, 5.1%)
	500 +	186	180	96.8% (93.1%, 98.5%)	3.2% (1.5%, 6.9%)
	Total	1341	1207	90.0% (88.3%, 91.5%)	10.0% (8.5%, 11.7%)
All CNVs		2187	1928	88.2% (86.7%, 89.4%)	11.8% (10.6%, 13.3%)
cnLOH	100-200	132	106	80.3% (72.7%, 86.2%)	19.7% (13.8%, 27.3%)
	200-500	102	99	97.1% (91.7%, 99.0%)	2.9% (1.0%, 8.3%)
	500 +	58	58	100.0% (93.8%, 100.0%)	0.0% (0.0%, 6.2%)
	Total	292	263	90.1% (86.1%, 93.0%)	9.9% (7.0%, 13.9%)

* The number of aberrations analyzed in each range bin, including indeterminate CNVs as "not confirmed".

Alternative assessment criteria were also considered for informational purposes. With the exception for more "indeterminate" calls, which are an expected consequence of using multiple comparators in parallel, the overall conclusion was not impacted.

In summary, the analytical performance of the GenetiSure Dx Postnatal Assay has been determined to be highly accurate and appropriate for its intended use as a source of reliable information contributing to a clinical diagnosis in subjects with potential genetic aberrations.

(b) Precision of Measurement

Reproducibility

The aim of the Reproducibility Study was to demonstrate that GenetiSure Dx Postnatal Assay (also referred to during development as the Molecular Copy Number Change (CNC) Test) achieves acceptable, reproducible results when performed at multiple laboratory sites by multiple operators over multiple days. Replicates of forty-eight (48) test samples were processed by two separate operators, at each of three individual clinical laboratories, in three (3) one-week intervals for a total of 864 data points.

The forty-eight (48) test samples were selected from cell-lines with a wide range of known aberrations (amplifications, deletions and copy neutral loss of heterozygosity (cnLOH)). The aberrations met the following criteria: common syndromes ('known syndromic regions'), analytically challenging regions, claimed minimal resolution, varying aberration size ranges, and genomic coverage of aberrations. Multiple samples had multiple aberrations spanning multiple criteria. Test sample selection criteria encompasses aberrations expected to be found in normal whole blood samples.

All individual aberrations reported within each processed test sample, regardless of expected pathogenicity, were compared to their respective replicates (18 replicates for each aberration, operator by site by week) by pairwise replicate analysis (PRA), requiring at least 50% overlap of chromosomal coordinates for confirmation. Positive agreement was assessed separately for small copy number variants (CNVs, 5-20 probes contained with the aberration), larger CNVs (>20 probes), or cnLOH regions. The results demonstrate that the pre-defined acceptance criteria were met for each category with a pairwise replicate agreement of 80.22%, 95.83%, and 89.08%, respectively. Using a more stringent 80% overlap criteria for pairwise replicate agreement, acceptance criteria were also met.

Data are further refined by size, probe number, aberration type, and study variable (e.g. operator, site, test sample). Alternative metrics of positive percent agreement, call rate, and breakpoint accuracy/endpoint deviation are presented.

To provide additional insight into the reproducibility of the test as a function of reported aberration size (in kb), the data were categorized into more refined size bins. The results demonstrated that when comparing all replicates of all test samples across all days, sites, and operators, using a 50% aberration overlap criteria, the overall pairwise replicate agreement across all sizes of amplifications and deletions was 85.0%. Pairwise replicate agreement across the various kb bins ranged from 75.9% to 100%. For copy number amplifications, the overall agreement was 85.7%; for deletions, the overall agreement was 84.6%. For LOH, the overall pairwise replicate agreement was 89.1%. Applying a more stringent 80% overlap criteria produced overall agreements of 82.3% for amplifications and deletions combined, 84.4% for amplifications, 81.3% for deletions, and 87.9% for LOH.

When assessing specifically the agreement between replicates for positive aberration calls by PPA analysis, the agreement was 89.3% for all copy number calls and 92.7% for LOH using the 50% overlap criteria.

Call rate averaged 78.1% for CNVs, and 74.9% for cnLOH.

Table 12. Reproducibility of Aberrations Categorized by Size (in kb) and Type

Aberration Type	Aberration Range (kb)	# Aberrations	Call Rate (%)	Pairwise Replicate Agreement (%)		PPA (%)	
				Overlap			
				50%	80%	50%	80%
AMP	**10 - 50**	5	51.2	82.5	82.5	82.9	82.9
	50 - 100	3	68.7	96.3	96.3	97.3	97.3
	100 - 200	13	50.5	79.9	79.8	80.1	80.0
	200 - 500	26	82.7	86.3	84.6	91.5	89.4
	500 - 1000	9	79.7	80.2	78.9	87.6	86.0
	1000 - 2000	7	72.1	90.7	82.8	92.4	81.6
	2000 - 5000	11	65.1	75.9	75.9	79.7	79.7
	5000 +	13	93.2	98.4	98.4	99.1	99.1
	Total	87	73.8	85.7	84.4	89.9	88.2
DEL	**10 - 50**	14	51.6	76.8	76.1	77.6	76.2
	50 - 100	2	100.0	100.0	89.5	100.0	89.5
	100 - 200	23	81.4	82.3	78.1	88.1	82.9
	200 - 500	31	82.6	81.8	75.5	86.3	78.7
	500 - 1000	55	72.8	81.2	76.2	85.2	78.5
	1000 - 2000	30	83.3	86.4	85.9	91.5	91.0
	2000 - 5000	18	88.9	87.4	85.1	89.9	87.3
	5000 +	20	100.0	100.0	100.0	100.0	100.0
	Total	193	80.1	84.6	81.3	89.0	84.8
All CNVs (AMP & DEL)	**Total**	280	78.1	85.0	82.3	89.3	85.8
cnLOH	**5000 - 10000**	21	50.6	77.1	76.8	77.4	76.8
	10000 - 20000	11	91.5	99.0	96.4	99.4	96.7
	20000 +	13	100.0	100.0	98.4	100.0	98.4
	Total	45	74.9	89.1	87.9	92.7	91.1

When results were binned by the number of probes in an aberration, rather than size in kb, using the 50% overlap criteria, the overall pairwise replicate agreement was similar to the above: 86.2% for combined amplifications and deletions (ranging from 70.6% to 100%), 86.1% for amplifications alone, 86.3% for deletions alone, and 89.1% for LOH.

Using the 80% overlap criteria, overall agreements were 84.6% for amplifications and deletions combined, 85.3% for amplifications, 84.2% for deletions, and 88.4% for LOH. PPA for the 50% overlap criteria was 90.9% and 92.7% for CNVs and cnLOH, respectively. Call rate averaged 78.1% for CNVs, and 74.9% for cnLOH.

Table 13. Reproducibility of Aberrations Categorized by Probe Number and Type

Aberration Type	Aberration Range (# Probes)	# Aberrations	Call Rate (%)	Pairwise Replicate Agreement (%)		PPA (%)	
				Overlap			
				50%	80%	50%	80%
AMP	**5 - 7**	11	38.0	76.5	76.5	69.0	69.0
	7 - 10	15	54.1	70.6	69.4	72.8	70.6
	10 - 15	23	87.9	89.4	88.3	94.0	92.7
	15 - 20	11	66.5	82.4	79.9	86.8	83.1
	20 - 30	9	89.6	97.5	97.5	97.9	97.9
	30 - 100	3	70.3	93.0	93.0	95.0	95.0
	100 - 500	3	72.3	90.2	90.2	93.2	93.2
	500 +	12	100.0	100.0	100.0	100.0	100.0
	Total	87	73.8	86.1	85.3	90.5	89.4
DEL	**5 - 7**	36	61.1	76.6	75.6	80.9	79.1
	7 - 10	39	65.5	77.6	75.4	82.8	79.6
	10 - 15	42	81.9	85.5	81.0	90.5	85.0
	15 - 20	18	96.9	95.9	94.0	97.6	95.7
	20 - 30	16	87.5	89.1	85.7	91.6	87.9
	30 - 100	10	92.2	93.2	92.9	96.3	96.0
	100 - 500	17	100.0	100.0	100.0	100.0	100.0
	500 +	15	100.0	100.0	100.0	100.0	100.0
	Total	193	80.1	86.3	84.2	91.1	88.5
All CNV (AMP & DEL)	**Total**	280	78.1	86.2	84.6	90.9	88.8
cnLOH	**100 to 200**	25	54.8	80.3	80.3	82.0	82.0
	200 to 500	13	100.0	100.0	97.7	100.0	97.7
	> 500	7	100.0	100.0	100.0	100.0	100.0
	Total	45	74.9	89.1	88.4	92.7	91.8

CONCLUSIONS: More refined categorization of aberrations by size and probe number, and further analyses of these confirmations using PPA and call rate calculations, support the reproducibility conclusions established for small (5-20

probes), large (>20 probes), and cnLOH categories by pairwise confirmation. In general, pairwise replicate agreement, PPA, and call rate increase with aberration size and probe number, although the aberration numbers within each bin varies.

Reagent and Scanner Precision

The aim of the Reagent Lot-Scanner Precision Study was to demonstrate that GenetiSure Dx Postnatal Assay (also referred to during development as the Molecular Copy Number Change (CNC) Test) achieves acceptable, precise results when performed using multiple reagent manufacturing lots and when analyzed on multiple scanner instruments. Duplicates of six (6) test samples containing a range of chromosomal aberrations (amplifications, deletions, and copy-neutral loss of heterozygosity (cnLOH)) were processed by one operator, using combinations of three (3) reagent lots analyzed on three (3) scanner instruments across five (5) processing weeks at a single site for a total of 108 data points.

Individual aberrations called within each processed test sample were compared to their respective replicates (18 replicates for each aberration, 2 replicates by 9 reagent/scanner combinations) by pairwise replicate analysis (PRA), requiring at least 50% overlap of chromosomal coordinates for confirmation. Agreement was assessed separately for small copy number variants (CNVs, 5-20 probes contained within the aberration), larger CNVs (>20 probes), or cnLOH regions. The results demonstrate that the pre-defined acceptance criteria were met for each category with a pairwise replicate agreement of 81.61%, 93.20%, and 86.93%, respectively. Results were similar when using a more stringent 80% overlap criteria for pairwise replicate agreement.

Data were further refined by size, probe number, aberration type, and study variable (e.g. reagent lot, scanner, processing week). Alternative metrics of positive percent agreement, call rate, and breakpoint accuracy/endpoint deviation are presented.

To provide additional insight into the precision of the test as a function of reported aberration size (in kb), the data were categorized into more refined size bins. The results demonstrated that when comparing all replicates of all test samples across all lots, scanners, and weeks, using a 50% aberration overlap criteria, the overall pairwise replicate agreement across all sizes of amplifications and deletions was 85.3%. Pairwise replicate agreement across the various kb bins ranged from 72.8% to 100%. For copy number amplifications, the overall agreement was 87.3%; for deletions, the overall agreement was 83.5%. For LOH, the overall pairwise replicate agreement was 86.9%. Applying a more stringent 80% overlap criteria produced overall agreements of 85.1% for amplifications and deletions combined, 87.3% for amplifications, 83.1% for deletions, and 82.2% for LOH.

When assessing specifically the agreement between replicates for positive aberration calls by PPA analysis, the agreement was 89.0% for all copy number calls and 92.1% for LOH using the 50% overlap criteria.

Call rate averaged 69.2% for CNVs, and 86.5% for cnLOH.

Table 14. Reproducibility of Aberrations Categorized by Size (in kb) and Type

Aberration Type	Aberration Range (kb)	# Aberrations	Call Rate (%)	Pairwise Replicate Agreement (%)		PPA (%)	
				Overlap			
				50%	80%	50%	80%
AMP	**10-200**	0	N/A	N/A	N/A	N/A	N/A
	200-1000	6	76.0	84.9	84.9	88.8	88.8
	1000 +	9	62.3	88.9	88.9	91.1	91.1
	Total	15	67.8	87.3	87.3	90.1	90.1
DEL	**10-200**	6	75.8	87.8	87.0	92.0	91.0
	200-1000	7	53.3	72.8	72.5	73.6	72.9
	1000 +	3	100.0	100.0	100.0	100.0	100.0
	Total	16	70.5	83.5	83.1	88.0	87.4
All CNVs (AMP & DEL)	**Total**	31	69.2	85.3	85.1	89.0	88.7
cnLOH	**5000-10000**	10	67.7	69.9	67.1	77.6	73.5
	10000-20000	8	100.0	99.8	90.0	99.8	90.0
	20000 +	6	100.0	98.1	97.1	98.1	97.1
	Total	24	86.5	86.9	82.2	92.1	86.6

When results were binned by the number of probes in an aberration, rather than size in kb, using the 50% overlap criteria, the overall pairwise replicate agreement was similar to the above: 85.9% for combined amplifications and deletions (ranging from 74.8% to 100%), 88.0% for amplifications alone, 83.9% for deletions alone, and 86.9% for LOH.

Using the 80% overlap criteria, overall agreements were 85.4% for amplifications and deletions combined, 87.3% for amplifications, 83.7% for deletions, and 83.7% for LOH. PPA for the 50% overlap criteria was 89.8% and 92.1% for CNVs and cnLOH, respectively. Call rate averaged 69.2% for CNVs, and 86.5% for cnLOH.

Table 15. Reproducibility of Aberrations Categorized by Probe Number and Type

Aberration Type	Aberration Range (# Probes)	# Aberrations	Call Rate (%)	Pairwise Replicate Agreement (%)		PPA (%)	
				Overlap			
				50%	80%	50%	80%
AMP	**5-10**	2	69.5	74.8	74.8	81.9	81.9
	10-20	6	55.7	89.8	87.9	90.8	87.5
	20 +	7	77.7	90.3	90.3	93.8	93.8
	Total	15	67.8	88.0	87.3	91.2	90.1
DEL	**5-10**	10	53.4	75.4	75.0	77.0	76.1
	10-20	3	98.0	96.3	96.3	98.1	98.1
	20 +	3	100.0	100.0	100.0	100.0	100.0
	Total	16	70.5	83.9	83.7	88.6	88.2
All CNVs (AMP & DEL)	**Total**	31	69.2	85.9	85.4	89.8	89.1
cnLOH	**100-200**	13	75.2	76.8	74.1	84.5	80.8
	200-400	7	100.0	98.2	92.2	98.2	92.2
	400 +	4	100.0	100.0	100.0	100.0	100.0
	Total	24	86.5	86.9	83.7	92.1	88.3

CONCLUSIONS: More refined categorization of aberrations by size and probe number, and further analyses of these confirmations using PPA and call rate calculations, support the conclusions regarding precision established for small (5-20 probes), large (>20 probes), and cnLOH categories by pairwise confirmation.

DNA Extraction Precision

The extraction precision study was performed to assess the aberration calling concordance of the GenetiSure Dx Postnatal Assay across repeated DNA extractions from the same blood sample performed by different operators in multiple runs, to determine repeatability and precision.

A panel of twenty-four (24) samples was tested by each operator. The gDNA from the samples was extracted using the same lot of the Qiagen kit, at one site, in duplicate, by 3 operators, on 3 separate days, for a total of 432 extractions (3 operators x 3 days x 2 duplicates x 24 samples). Each of the three operators labelled a set of 48 extracted samples (24 different samples x 2 extractions) per week for 3 weeks for a total of 144 test results run per operator over the course of the study. The extracted gDNA replicates were tested in the GenetiSure Dx Postnatal Assay in 3 weeks, each corresponding to a specific day of extraction.

Primary analysis was performed using pairwise comparison of aberration results on each of the 18 replicates (3 operators x 3 days x 2 duplicates) for each sample. An aberration was considered confirmed if at least 50% of the region of aberration overlapped between the replicates being compared.

The results of the 50% overlap analysis demonstrated that results obtained from multiple extractions of the same sample were highly concordant, regardless of operator and day upon which the samples were extracted. The individual Pairwise Replicate Agreement % values stratified by week or operator were similar to each other and to the overall averages shown in the table below (82.17% for copy number variants (CNVs) called by 5-20 probes, 98.47% for CNVs called by >20 probes, and 81.15% for copy-neutral loss of heterozygosity (cnLOH)), which further supports that similar assay performance can be expected from different extractions, personnel, days, and samples.

Table 16. Pairwise Replicate Agreement Based on 50% Overlap of Base Pairs

Category	Aberration Type	Unique Aberrations	Pairwise Confirmed	Number of Comparisons	Percent Confirmed	Acceptance Criteria
5 to 20	AMP	36	4326	5391	80.24	NA
5 to 20	DEL	46	5817	6953	83.66	NA
5 to 20	AMP or DEL	82	10143	12344	82.17	70
>20	AMP	29	4244	4387	96.74	NA
>20	DEL	33	4932	4932	100.00	NA
>20	AMP or DEL	62	9176	9319	98.47	80
--	cnLOH	16	1959	2414	81.15	NA

7.2.1.1 Analytical Sensitivity

Limit of Detection – DNA Input

To determine the analytical sensitivity, or the Limit of Detection (LOD) of the GenetiSure Dx Postnatal Assay, also referred to as the Agilent Molecular CNC (Copy Number Change) Test, this study was conducted to evaluate the minimum and maximum amounts of genomic DNA (gDNA) acceptable as the assay input to detect copy number variations (CNVs) and copy neutral loss of heterozygosity (cnLOH) accurately. Twenty-four (24) gDNA samples with known chromosomal aberrations were obtained from Coriell Institute for Medical Research (Coriell). These DNA samples were tested in the assay using two (2) lots of reagents across a range of varied DNA input levels from 0.125 µg (125 ng) to 1 µg (1000 ng), with 0.5 µg (500 ng) as the recommended input quantity (standard).

The study assessed the impact of various gDNA input on aberration calling and determined the upper and lower limits of detection (ULOD and LLOD) of the assay by comparing the percentage of aberrations confirmed at each non-standard DNA input level against pre-defined acceptance criteria. Data from this study support the use of 500 ng as the recommended input amount. The study data, and supplemental data generated under similar study conditions, demonstrate that performance does not decline down to 375 ng. The data support a conservative LLOD at 375 ng and a common ULOD at 1000 ng for both copy number and cnLOH aberrations. For copy number aberrations only, the LLOD could be further reduced to 250 ng.

The assay performs robustly at the recommended input amount of 500 ng and is stable at considerably lower and higher amounts.

Limit of Detection – Mosaicism

To determine the sensitivity (LoD) of the Agilent Molecular CNC (Copy Number Change) Test for the detection of mosaic cytogenetic abnormalities, aberrant cell line DNAs containing known copy number changes were mixed with a reference background DNA in different percentages to mimic various levels of mosaicism. The admixtures were analyzed for the presence of the copy number changes detectable in the pure aberrant cell line DNAs. Secondary analysis addressed the rate of false positive calls present in admixtures as compared to the pure reference sample.

Large copy number aberrations could be reliably detected when present in a 50% or greater admixture. Some aberrations were correctly identified at lower than a 50% level, but the sensitivity of detection was reduced. Results were similar for both amplifications and deletions. Smaller aberrations could not be reliably detected in any of the admixtures. Despite the reduced sensitivity, the specificity of aberrations called remained largely unaffected across all levels of mosaicism evaluated and both size ranges.

The results demonstrate that the GenetiSure Dx Postnatal Assay has the sensitivity to detect large mosaic amplifications and deletions down to the 50% level.

7.2.1.2 Analytical Specificity

Interfering Substances

To determine the effects of interfering substances on the results of the GenetiSure Dx Postnatal Assay, the study evaluated the impact of hemoglobin, conjugated bilirubin, unconjugated bilirubin and triglycerides (triolein) spiked into whole blood prior to gDNA isolation.

Blood drawn from twelve (12) phenotypically normal males and twelve (12) phenotypically normal females was used in the testing.

The list of aberrations for each sample containing a given interferent was reported and compared with the 'non-adulterated control' list for the same sample. An aberration was considered confirmed if the test result identified a region of aberration that overlaps between the sample and control by at least 50%. The test was considered robust to a given interferent when 75% of the amplifications/deletions in the 5-20 probe category and 90% of the amplifications/deletions in the >20 probe category in the 'non-adulterated control' were confirmed.

An additional analysis was also performed at 80% overlap between regions. The results of both the 50% and 80% overlap analysis methods demonstrated that the test results are not altered by the presence of excessive hemoglobin, triglycerides, or bilirubin (conjugated or unconjugated) in the patient whole blood specimen.

Cross Contamination

The GenetiSure Dx Postnatal Assay consists of a glass slide composed of four (4) independent microarrays that are sealed by a gasket slide during sample hybridization. Cross-contamination can arise during sample processing, especially during hybridization set-up, when samples are loaded onto each of the 4 adjacent

arrays or during the overnight incubation due to gasket leakage. The presence of contamination can result in corrupt and inaccurate patient data.

This study was designed to determine if cross contamination occurs during the routine GenetiSure Dx Postnatal Assay workflow and, if so, what the impact on data would be. For this study, two (2) male and two (2) female Coriell DNA samples, each with distinctive sets of known chromosomal aberrations, were tested across multiple microarray slides under conditions that would either allow or prevent detection of cross contamination between the adjacent arrays on the slides. Four (4) microarray slides served as the "non-contaminated condition" with four replicates of the same sample placed on each of the four arrays of the slides. Six (6) slides served as a test for "potential cross-contamination" that could occur between adjacent arrays within a single slide during the hybridization set–up or overnight incubation. For these slides, the sample replicates were alternated on the slide with sample replicates from a different sample. The copy number variation (CNV) and copy-neutral loss of heterozygosity (cnLOH) aberration results from the "potential cross-contamination" microarray slides were compared to the aberration results from the "non-contaminated condition" microarray slides, using a 50% overlap criteria, to determine if detectable cross contamination had occurred on the test slides. Additionally, gasket-related cross-contamination was evaluated by use of three (3) different lots of gasket slides.

The 50% overlap analysis demonstrated that results obtained were highly concordant between the aberrations detected in both the "non-contaminated condition" and the "potential cross-contamination condition", easily meeting the established acceptance criteria, which were established based on the tested condition not impacting the detection of aberrations in a given sample. No suspected cross contamination was detected. This supports the appropriateness of the sample handling workflow, the accuracy of the data collected, and the integrity of the gasket slide materials for use with the GenetiSure Dx Postnatal Assay.

7.2.2 Clinical Performance

Eight hundred (800) samples from patients suspected of having pathogenic aberrations (SPA samples) were processed utilizing the GenetiSure Dx Postnatal Assay. The samples had been collected from three (3) regionally distinct clinical institutions that offered postnatal array testing for the detection of chromosomal abnormalities. One hundred (100) samples from phenotypically normal individuals were also processed using the GenetiSure Dx Postnatal Assay and were used to assess the aberrations that might be expected to be found in a normal (non-patient) population.

The aberrations detected in each sample, for all nine hundred (900) samples, were interpreted by one of four cytogeneticists as Benign, Likely Benign, Variant Of Unknown Significance (VOUS), Likely Pathogenic, or Pathogenic.

The test results, per sample, were compared to historical array data from the respective collection site, which were generated using the methods established at each laboratory.

All reported Pathogenic and Likely Pathogenic copy number variants (CNVs), amplifications and deletions, were subject to confirmation by alternative methods:

i. Confirmation data from the sample collection site was reviewed. If prior confirmation data, from the collection sites, was available (e.g., quantitative polymerase chain reaction (qPCR), fluorescence *in situ* hybridization (FISH) or karyotype), it was considered adequate, and no additional confirmation test was performed.
ii. Remaining aberrations were assessed by qPCR assays for confirmation per pre-determined guidelines. Briefly, at least 1 qPCR CNV confirmatory assay was required within each target genomic region (copy number aberration interval). For larger regions (>400 kb), more than 1 qPCR assay was preferred, ideally with qPCR assays distributed across the target region, if possible. The actual number of qPCR assays selected was in part determined by the availability of predesigned assays in a given target region and the quantity of DNA available for analysis. Assays were selected from Predesigned TaqMan® Copy Number Assays from Applied Biosystems (Thermo Fisher Scientific, Cat. # 4400291). Aberrations without any assays available in the region or without sufficient DNA were excluded from qPCR confirmation.

In routine clinical practice, reported Pathogenic and Likely Pathogenic copy-neutral loss of heterozygosity (cnLOH) aberrations are not subject to analytical confirmation. Clinical follow up (such as parental, methylation and/or sequencing studies to understand the mechanism or impacts of the cnLOH) is conducted. Such follow up was beyond the scope of this study, and hence was not performed. The lack of clinical follow up on these aberrations does not impact the data interpretation for this study or the outcome of the study.

Study Results

The diagnostic yield for the GenetiSure Dx Postnatal Assay, when considering only copy number aberrations, was 15%. This increased to 20% when cnLOH aberrations were also considered.

Table 17. Diagnostic Yield by Collection Site (95% CI)

Collection Site	Number of Samples	Collection Site: Number of Pathogenic Calls	Collection Site: Diagnostic Yield	GenetiSure Dx Postnatal Assay: Number of Pathogenic Calls	GenetiSure Dx Postnatal Assay: Diagnostic Yield
Copy Number Aberrations Only					
Site 1	257	29	11% (8.0%, 15.7%)	39	15% (11.3%, 20.1%)
Site 2	313	35	11% (8.2%, 15.2%)	33	11% (7.6%, 14.4%)
Site 3	230	48	21% (16.1%, 26.6%)	45	20% (15.0%, 25.2%)
TOTAL	800	112	14% (11.8%, 16.6%)	117	15% (12.3%, 17.2%)
All Aberrations (Copy Number and cnLOH)					
Site 1	257	29	11% (8.0%, 15.7%)	48	19% (14.4%, 23.9%)
Site 2	313	39	12% (9.2%, 16.6%)	60	19% (15.2%, 23.9%)
Site 3	230	48	21% (16.1%, 26.6%)	51	22% (17.3%, 28.0%)
TOTAL	800	116	15% (12.2%, 17.1%)	159	20% (17.3,22.8%)

When considering only copy number aberrations, diagnostic yields were comparable between the collection sites and the GenetiSure Dx Postnatal Assay; however, differences were seen among the three collection sites, with Site 3 having a higher diagnostic yield than either Site 1 or Site 2. As the results from the GenetiSure Dx Postnatal Assay show the same trend across collection sites, this difference is likely due to diversity in the population of patients referred to the three sites, rather than a difference in the interpretation of aberrations, as the same set of cytogeneticists assessed the GenetiSure Dx Postnatal Assay aberrations, irrespective of the collection site.

The non-pathogenic category is encompassed by samples with interpretations of VOUS, Likely Benign, Benign, or those with no aberrations reported. Results of the PPA and NPA analysis are presented considering only copy number aberrations or considering both copy number and cnLOH aberrations. For the copy number aberration only analysis, samples with pathogenic cnLOH aberrations were considered as non-pathogenic, unless they also included a pathogenic copy number aberration.

Table 18. Comparison of Sample Classification between the Collection Site and the GenetiSure Dx Postnatal Assay, Considering only Copy Number Aberrations

		Collection Site Aberration Interpretation					
		Pathogenic Interpretation		Non-Pathogenic Interpretation			
GenetiSure Dx Postnatal Assay Interpretation		Pathogenic	Likely Pathogenic	VOUS	Likely Benign[1]	Normal[2]	**Total**
	Pathogenic	56	14	9	0	3	82

Pathogenic Interpretation	Likely Pathogenic	12	4	11	0	8	35
Non-Pathogenic Interpretation	VOUS	5	8	35	0	32	80
	Normal[2]	6	7	80	1	509	603
Total		79	33	135	1	552	800
PPA[3]		86/112 = 76.8% (95%CI=68.2%-83.6%)					
NPA[4]		657/688 = 95.5% (95%CI=93.7%-96.8%)					

[1]One Site 2 sample was presented with the interpretation on Likely Benign.
[2]As described in the study report, samples from the GenetiSure Dx Postnatal Assay or Site 1 with either only Benign or Likely Benign aberrations, or samples without aberrations are classified as "Normal". Site 3 and Site 2 provided sample classification of "Normal".
[3]Positive Percent Agreement (PPA): Percent [(Agilent GenetiSure Dx Postnatal Assay = Pathogenic & Collection site classification = Pathogenic)/(Collection site classification = Pathogenic)]
[4]Negative Percent Agreement (NPA): Percent [(Agilent GenetiSure Dx Postnatal Assay = Non-pathogenic & Collection site classification = Non-Pathogenic)/(Collection site classification = Non-Pathogenic)]

When considering only copy number aberrations in the sample classification, PPA was 76.8% and NPA was 95.5%. In total, twenty-six (26) samples which were determined to have Pathogenic or Likely Pathogenic copy number aberrations by the collection sites were reported as non-pathogenic by the GenetiSure Dx Postnatal Assay. As outlined in the study report, most of these aberrations were either detected by GenetiSure Dx Postnatal Assay, but interpreted differently by the cytogeneticist, or below the detection limit of the Assay.

Table 19. Comparison of Sample Classification between the Collection Site and the GenetiSure Dx Postnatal Assay, Considering Copy Number and cnLOH Aberrations

		Collection Site Aberration Interpretation					
		Pathogenic Interpretation		Non-Pathogenic Interpretation			
GenetiSure Dx Postnatal Assay Interpretation		Pathogenic	Likely Pathogenic	VOUS	Likely Benign[1]	Normal[2]	**Total**
Pathogenic Interpretation	Pathogenic	56	14	9	0	3	82
	Likely Pathogenic	14	5	23	0	35	77
Non-Pathogenic Interpretation	VOUS	5	10	59	0	46	120
	Normal[2]	7	5	74	1	434	521
Total		82	34	165	1	518	800
PPA[3]		89/116 = 76.7% (95%CI=68.3%-83.5%)					
NPA[4]		614/684 = 89.8% (95%CI=87.3%-91.8%)					

[1]One Site 2 sample was presented with the interpretation on Likely Benign.
[2]As described in the study report, samples from the GenetiSure Dx Postnatal Assay or Site 1 with either only Benign or Likely Benign aberrations, or samples without aberrations are classified as "Normal". Site 3 and Site 2 provided sample classification of "Normal".
[3]Positive Percent Agreement (PPA): Percent [(Agilent GenetiSure Dx Postnatal Assay = Pathogenic & Collection site classification = Pathogenic)/(Collection site classification = Pathogenic)]

[4]Negative Percent Agreement (NPA): Percent [(Agilent GenetiSure Dx Postnatal Assay = Non-pathogenic & Collection site classification = Non-Pathogenic)/(Collection site classification = Non-Pathogenic)]

When considering all aberrations, PPA remained similar at 76.7%, and NPA dropped to 89.8%, which is consistent with the higher diagnostic yield for the GenetiSure Dx Postnatal Assay when considering all aberrations. Twenty-seven (27) samples were called as Pathogenic at the collection sites and non-pathogenic by the GenetiSure Dx Postnatal Assay.

As per the SPA samples, data obtained for the phenotypically normal samples were aggregated at the sample level. Results of the sample level analysis are shown in the table below.

Table 20. Phenotypically Normal Individual Sample Level Summary

Interpretation	Number of Samples
Normal (all Likely Benign, Benign, or no aberrations identified)	78
VOUS	14
Likely Pathogenic	5
Pathogenic	3
Total	**100**

In total, eight (8) of the 100 samples had aberrations classified as Likely Pathogenic or Pathogenic, the details of those aberrations are listed below.

Table 21. Pathogenic and Likely Pathogenic Aberrations Detected in Phenotypically Normal Samples

Sample ID	Type	Size	Chr Band	Interpretation	Confirmation Method	Confirmation Result
CLIS-CMC-0033-F	AMP	100 kb	22q13.33	Pathogenic	qPCR	Unconfirmed
CLIS-CMC-0216-F	DEL	18.2 kb	16q24.3	Pathogenic	qPCR	Confirmed
CLIS-CMC-0253-M	AMP	646 kb	Xp21.2-p21.1	L. Pathogenic	qPCR	Confirmed
CLIS-CMC-0259-F	LOH	20 Mb	2q14.3-q22.3	L. Pathogenic	N/A	N/A
CLIS-CMC-0281-F	DEL	13.6 kb	15q11.2	L. Pathogenic	qPCR	Confirmed
CLIS-CMC-0372-M	LOH	11 Mb	6q23.3-q25.1	L. Pathogenic	N/A	N/A
CLIS-CMC-0497-M	AMP	59 Mb+93 Mb	Xp + Xq	Pathogenic	qPCR	Confirmed
CLIS-CMC-0507-F	AMP	1.5 Mb	16p13.11	L. Pathogenic	qPCR	Confirmed

Of the eight (8) samples with Pathogenic or Likely Pathogenic aberrations reported, two (2) of those were cnLOH aberrations reported as Likely Pathogenic. The other six (6) samples contained copy number changes, five (5) of which were confirmed by qPCR. One (1) of the copy number changes, a 100 kb amplification on Chromosome 22, was not confirmed by qPCR. Of note, one of the samples was identified as containing an additional X chromosome (Karyotype 47, XXY), which is commonly associated with Klinefelter Syndrome (https://ghr.nlm.nih.gov/condition/klinefelter-syndrome).

PPA and NPA analysis revealed strong correlation with previous clinical data, especially for copy number changes. Addition of cnLOH aberrations in the comparison did not significantly impact the PPA, but the reduced NPA is consistent with the fact that few cnLOH were reported as pathogenic by the collection sites.

7.3 Stability (Excluding Specimen Stability)

All QC metrics pass for all time points tested, with no overlap of the 95% confidence intervals with any of the QC metric acceptance criteria for all time points. In addition, stability duration based on the observed time trend, where calculated, is beyond the 18-month time point for all QC metrics. Shelf life stability is therefore established for minimally eighteen months.

7.3.1 Claimed Shelf-Life

Catalog Number	Product Name	Targeted Kit Shelf Life
K1201A	GenetiSure Dx Postnatal Assay	18 months

Shelf life is assigned on a batch/lot basis. If a kit component has a shorter shelf life than the targeted kit shelf life then the kit shelf life is limited by the shelf life of the component.

7.3.2 In-Use Stability

All QC metrics pass for all storage times and conditions tested for all components and reaction intermediates, with no overlap of the 95% confidence intervals with any of the QC metric acceptance criteria for all conditions. Stability is established for opened microarray packaging for up to 60 days.

7.3.3 Shipping Stability

All QC metrics pass for all time points tested to date, with no overlap of the 95% confidence intervals with any of the QC metric acceptance criteria for all time points. In addition, stability duration based on the observed time trend, where calculated, is well beyond the 18-month time point for all QC metrics. Stability after transport is therefore established for minimally eighteen months.

7.3.4 Specimen Stability

Blood specimens can be stored at 2°C to 8°C for up to 7 days.

Extracted gDNA may be stored at 2°C to 8°C for up to one year and at –15°C to –25°C for storage greater than one year.

7.4 Software Verification and Validation

Testing of the CytoDx software application was governed by the Software Test Plan: Test Plan CytoDx. The plan was based on the IEEE Std 829-1998 Standard for Software Test Documentation.

The objective of the test effort was to verify that the software requirements were satisfied by execution of the defined test plans. Testing was performed for progression (testing new and corrected features) and regression (testing that no unintended changes have occurred)

Verification testing was performed using 18 subordinate test procedures:

- CytoDx_3.1_PerformanceRequirement_TP
- CytoDx_3.2_Usability_TP
- CytoDx_3.3_Labelling_TP
- CytoDx_3.4_Supportability_TP
- CytoDx_3.5_Platform_TP
- CytoDx_3.6_UserInterface-Global_TP

- CytoDX_3.7_Analysis Workflow_TP
- CytoDx_3.8_Review Workflow_TP
- CytoDx_3.9_Sample Review - All Samples_TP
- CytoDx_3.10_SampleReview-Triage_TP
- CytoDx_3.11_MultiSampleView_TP
- CytoDx_3.12_ReportingAuditingLogging_TP
- CytoDx_3.13_PrimaryAnalysis_TP
- CytoDx_3.14_SecondaryAnalysis_Algorithms_TP
- CytoDx_3.15_QC Metrics_TP
- CytoDX_3.16_Additional Documentation_TP
- CytoDX_ 3.17_Feature Extraction and Image View Specifications_TP
- CytoDX_3.18_Miscellanous_TP

Requirements (progression) testing was performed at either a component, or system level, as determined by the requirement being verified.

Regression testing was performed at a system level to insure conformity and compatibility with the historical system state, and to insure that no unintended changes had occurred during development.

System level testing was performed using G2600D SureScan instruments in lieu of G5761A SureScan Dx instruments. The two instruments are functionally equivalent for the purpose of testing. Only the instrument colors and final labeling are different between the two models. These differences do not affect instrument performance for the purpose of software testing.

Test results were captured for each test procedure including objective evidence.

Note that software to make clinical interpretations of results is NOT a component of the device. The interpretation and appropriate clinical reporting of findings falls within the practice of medicine.

7.5 An overall summary of the performance and safety

GenetiSure Dx Postnatal Assay is an aCGH or CMA platform that measures CNVs and cnLOH aberrations across a patient's genome. This is in contrast to other methods that detect aberrations in a specific region of the genome and/or at a lower resolution.

The diagnostic yield for the GenetiSure Dx Postnatal Assay, when considering only copy number aberrations, was 15%. This increased to 20% when cnLOH aberrations were also considered. This diagnostic yield is substantially higher when compared to other karyotyping methods such as G-banding.

The GenetiSure Dx Postnatal Assay was designed, manufactured, transported and stored in keeping with the biological characteristics of the platform and with minimal risk to the patient as well as the user. The clinical study results of the Assay demonstrated the intended performance was achieved without any user reported events. All known and foreseeable risks

were mitigated to an acceptable level. The benefit of utilizing the Assay for its intended purpose outweighs the risks.

7.6 Ongoing or planned post-market clinical follow-up

To date there are no post-market clinical follow-up data nor any clinical data from post-market surveillance.

8 Metrological traceability of assigned values

Not applicable to this device. The GenetiSureDx Postnatal Assay does not utilize calibrator or control materials.

9 Suggested profile and training of users

There are two levels of education and training of the intended users.

Medical Technologist or a health care professional skilled in testing and analyzing blood, tissue and other bodily fluid samples

- Agilent's mandatory wet-lab training to process the GenetiSure Dx Postnatal Assay safely and successfully
- Agilent's mandatory CytoDx software training to enter the data generated for analysis by software and for interpretation by the board certified Clinical Cytogenetics

Healthcare Professional board certified Clinical Cytogenetics or Molecular Genetics

- Agilent's mandatory CytoDx software training for use in documenting their interpretation

10 Risks and warnings

10.1 Residual risks and undesirable effects

The GenetiSure Dx Postnatal Assay does not have any significant residual risks nor any undesirable side-effects.

A System Level Failure Mode and Effects Analysis (Design FMEA) was performed on the Agilent K1201A GenetiSure Dx Postnatal Assay design and all potential risks have been identified and evaluated to the best of the team's knowledge.

The Risk Assessment was performed considering the intended use of the IVD kit as defined by the Instructions for Use (IFU). The FMEA analysis included the Postnatal Assay workflow as defined in the IFU.

In conducting the FMEA it was assumed that the Postnatal Assay was being used in accordance with the Intended Use and not being used off-label. The practice of the art of medicine was not included as part of the FMEA.

The results of the Postnatal Assay require interpretation by a Board Certified Cytogeneticist, Molecular Geneticist, Molecular Pathologist or similarly qualified clinician. That interpretation is outside the scope of the kit.

This FMEA did not cover the microarray scanner, which is covered by the SureScan Dx risk assessment.

Risks mitigated by changes to documentation (Notes/Warnings) did not receive a reduction in RPN from those mitigations. While the risk score did not reduce, the risks were considered to be mitigated as far as possible.

> Based upon EN ISO 14971:2019, a warning alone is not a valid control measure, but a warning can be used to inform the user of any residual risk remaining for the device. Other controls, i.e., inherent safe design or protective measures, are needed to be able to claim risk reduction. If a warning is applied directly to the device, any claim of risk reduction must be supported by data from usability or user studies. The use of a warning alone would not reduce probability or detectability without evidence (data) that it does so.

Some risks were mitigated through a functional change to the CytoDx software. As a result of more stringent usage of the QC metrics within the SW, risks were able to have their post-mitigation severity reduced when the fundamental nature of the risk, after the CytoDx change, would change from delivering an incorrect result to delivering a delayed result. Those risks are noted in the RAMM with an explanation in their comments field.

Hazards (Injury Risks)

The GenetiSure Dx Postnatal Assay is a microarray based diagnostic test used for detection of copy number variations (CNV) and copy-neutral loss of heterozygosity (cnLOH) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counseling, as appropriate.

The assay is intended for use in a clinical laboratory by trained laboratory professionals governed by the safety practices established for the laboratory in accordance with local regulations and relevant professional standards.

Assessment of common laboratory hazards are out of scope for the Failure Mode and Effects Analysis.

Version 6.0 of the RAMM identified three injury related risks associated with the GenetiSure Dx Postnatal Assay.

RiskID	Potential Hazard Description	Severity	Probability	Detection	RPN
P3	User fails to exercise suitable care with hazardous components (dye, hyb buffer), resulting in accidental exposure and injury	2	1	5	10
A5	Damaged or broken microarray or gasket slide results in injury to user, prior to use.	2	1	2	4
A6	Damaged or broken microarray or gasket slide results in injury to user, after sample is applied.	3	1	3	9

None of the three risks required assay specific mitigations after evaluation. These risks are covered by standard laboratory safety procedures and good laboratory practices. Warnings are present in GenetiSure Dx Postnatal Assay IFU and do not require modification.

Acceptability of Residual Risk

The GenetiSure Dx Postnatal Assay does not have any significant residual risks nor any undesirable side-effects. All known and foreseeable risks were mitigated to an acceptable level. The benefit of utilizing the Assay for its intended purpose outweighs the residual risks identified.

Individual risks that remain INV or INT after all risk control measures have been applied are listed in the table below. Justification for acceptability is documented after evaluating their acceptability using the acceptance criteria established in the Risk Management Plan, taking into account the state of the art where applicable.

Risks that remain after all risk control measures have been applied and/or after risk is considered to be reduced as far as possible and accepted after weighing benefits versus risk (benefit-risk analysis).

The summary of risk analysis activities demonstrates that the intended clinical benefit(s) is achieved, that the product is safe and effective under normal conditions of use/when used as intended. All risks were determined to be acceptable as the assay is intended for use in a clinical laboratory by trained laboratory professionals governed by the safety practices established for the laboratory in accordance with local regulations and relevant professional standards.

Patients are tested based upon pre-existing clinical presentation, and assay results are used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counseling, as appropriate.

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
H4	Sub optimal hybridization (too short, too long, wrong speed or temp)	**18**	**INV**	This is acceptable as documentation was updated but standard does not allow for any resulting reduction in risk scoring, SW changes related to QC metrics flagging causes delay in Dx, rather than incorrect result.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
H5	User uses improperly cleaned dished for microarray washing, resulting in wash artifacts	**18**	**INV**	This is acceptable as documentation was updated but standard does not allow for any resulting reduction in risk scoring, SW changes related to QC metrics flagging causes delay in Dx, rather than incorrect result.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
H7	Equipment failure: Oven, unnoticed by user	**18**	**INV**	This is acceptable as documentation was updated but standard does not allow for any resulting reduction in risk scoring, SW changes related to QC metrics flagging causes delay in Dx, rather than incorrect result.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
L13	User over-dries sample after purification, resulting in insufficiently resuspended sample, and too little labeled DNA added	**18**	**INV**	This is acceptable as documentation was updated but standard does not allow for any resulting reduction in risk scoring, SW changes related to QC metrics flagging causes delay in Dx, rather than incorrect result.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
L19	User specific differences in DNA quantitation (e.g., reagent lot) lead to a mismatch in reference and test DNA input, resulting in poor quality data	**18**	**INV**	This is acceptable as documentation was updated but standard does not allow for any resulting reduction in risk scoring,.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
H12	Hyb mix made incorrectly, resulting in sub-optimal hyb performance	**18**	**INV**	This is acceptable as documentation was updated but standard does not allow for any resulting reduction in risk scoring, SW changes related to QC metrics flagging causes delay in Dx, rather than incorrect result.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
A10	Users mistakenly applies incorrect sample to the array, resulting in a different patient identifier being associated with results	**32**	**INV**	Warnings and documentation changes implemented cannot be used to reduce risk score. Primary mitigation is through institutional procedures within the laboratory.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
L21	User mixes up adjacent samples during blood extraction or labelling reaction set up	**32**	**INV**	Warnings and documentation changes implemented cannot be used to reduce risk score. Primary mitigation is through institutional procedures within the laboratory.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
So02	Technician inputs inaccurate sample information	**24**	**INV**	Primary mitigation is through institutional procedures within the laboratory. Added mitigation of providing instruction on creating and importing a SAF, which should further minimize typographical errors.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
So05	Gene tracks and other annotation out of date, resulting in incorrect assignment of syndrome by cytogeneticist.	**24**	**INV**	Primary mitigation is through institutional procedures within the laboratory. Added mitigation by providing warning in IFU to check for updated track information on a regular basis as part of laboratory procedures (documentation).	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
So06	Information available in link-outs is inaccurate.	**40**	**INV**	Cytogeneticists are responsible for the quality/correctness of the reference information they use in the practicing of their art. Warning message on search links table reminding lab director to verify links.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
So13	FE algorithms produce inaccurate result; QC metrics do not indicate failure.	**16**	**INV**	Reporting is based upon the review and interpretation of a trained cytogeneticist. Abnormal artifacts present in the data would be an indication that the data may be faulty, and the sample should be rerun.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
So14	Imperfect slide image, results in undetectable inaccurate FE result	**16**	**INV**	Reporting is based upon the review and interpretation of a trained cytogeneticist. Abnormal artifacts present in the data would be an indication that the data may be faulty, and the sample should be rerun.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
So15	Aberration calling algorithm produces inaccurate result	**16**	**INV**	Reporting is based upon the review and interpretation of a trained cytogeneticist. Abnormal artifacts present in the data would be an indication that the data may be faulty, and the sample should be rerun.	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

Risk ID #	Brief Description of the Risk	Risk Priority Number (RPN)	INV or INT	Justification for Acceptability	Conclusion (Check One)
So16	No Tiff image analysis failure observed but inaccurate results produced due to other software defects	**16**	**INV**	Reporting is based upon the review and interpretation of a trained cytogeneticist. Abnormal artifacts present in the data would be an indication that the data may be faulty, and the sample should be rerun	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)
So20	"Silent" data corruption	**16**	**INV**	Reporting is based upon the review and interpretation of a trained cytogeneticist. Warning added to IFU that abnormal artifacts present in the data would be an indication that the data may be faulty, and the sample should be rerun	☒ Risk is acceptable ☐ Risk is unacceptable (Further actions required to make risk acceptable – List required actions in Section 2.6)

10.2 Warnings and precautions

Warnings
10X Oligo aCGH Blocking Agent (5190-7319) WARNING. Contains:
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride. Causes skin irritation. May cause respiratory irritation. Causes serious eye irritation. Wear protective gloves. Wear eye or face protection. Avoid breathing dust. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or physician if you

fell unwell. Store locked up. Dispose of contents and container in accordance with all local, regional, national and international regulations.

2X CGH Hybridization Buffer (5190-7320) DANGER. Contains: 4-Morphonlineethanesulfonic acid, monohydrate; Lithium chloride; lithium dodecyl sulphate. Causes skin irritation. Causes serious eye damage. Wear protective gloves. Wear eye or face protection. Wash hands thoroughly after handling. IF IN EYES: Rinse cautiously with water for several minutes. Immediately call a POISON CENTER or physician.

Precautions
1 For In Vitro Diagnostic Use
2 Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29- A. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 70% ethanol in deionized or distilled water.
3 This assay is for use only with gDNA extracted from human blood collected in the anticoagulant EDTA.
4 Wear appropriate personal protective equipment (PPE) – including disposable gloves, laboratory coat, and eye protection – when working in the laboratory or when handling specimens and reagents.
5 Do not pipette by mouth.
6 Do not eat, drink or smoke in laboratory work areas.
7 Wash hands thoroughly after handling specimens and reagents. Avoid contact of these materials with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated.
8 If spills of these reagents occur, dilute with water before wiping dry.
9 Material Safety Data Sheets (MSDS) are available from the Agilent website at:
www.chem.agilent.com/en- US/Search/Library/Pages/MsdsSearch.aspx.
10 To prevent contamination of reagents by nucleases, always wear powder- free laboratory gloves, and use dedicated solutions and *pipettors with nuclease- free, aerosol- barrier tips.*

10.3 Field safety corrective action

There are no additional aspects of safety to be mentioned and there have not been any FSCA or FSN in relation to the GenetiSure Dx Postnatal Assay.

11 Revision Log

SSP revision number	Revision date	Change description	Revision validated by the Notified Body
1.0	December 2020	Initial Version	☐ Yes *Validation language: English* ☒ No
2.0	March 2022	Updated Section 10 Risks to align with changes due to migration of RAMM to new template	☐ Yes *Validation language: English* ☒ No
3.0	June 2023	Updated revision log column 'Revision validated by the Notified Body' checkbox changed to Yes	☒ Yes *Validation language: English* ☐ No

4.0	October 2023	The summary of updates to the "SSP- GenetiSure Dx Postnatal Assay IVDR Registration.pdf" are listed below: • Section 1.2 References: Updated table to add reference to document number for the Agilent CytoDx Installation & Set Up Guide. • Section 2 Scope: Updated to add reference to CytoDx Software. • Section 3 General Device Information: Updated table and added Basic UDI-DI for the CytoDx Software. • Section 4.1 Intended purpose/Intended use. The CytoDx Software is already referenced as being used with the GenetiSure Dx Postnatal Assay. The Section 4.2 Indications and intended patient population contains the analysis results provided by the CytoDx Software as "intended for the postnatal detection of copy number variations (CNV) and copy- neutral loss of heterozygosity (cnLOH) in genomic DNA" • Section 5.1 –	☒ Yes *Validation language: English* ☐ No

SSP revision number	Revision date	Change description	Revision validated by the Notified Body
		Description of the device. The updated section 5.1 has description for CytoDx Software.	
5.0	March 2024	Updated document owner as Agilent Technologies, Inc. For Revision 4.0: Updated revision log column 'Revision validated by the Notified Body' checkbox changed to Yes.	☐ Yes *Validation language: English* ☒ No
6.0	April 2024	Removed "Confidential" from document footer since SSP is made publicly available per IVDR 2017/746.	☐ Yes *Validation language: English* ☒ No