DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

August 11, 2017

Agilent Technologies, Inc.
Bill Kurani, Ph.D.
Director of Regulatory Affairs and Quality Assurance
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

Re: K163367
Trade/Device Name: GenetiSure Dx Postnatal Assay
Regulation Number: 21 CFR 866.5920
Regulation Name: Postnatal chromosomal copy number variation detection system
Regulatory Class: Class II
Product Code: PFX
Dated: July 11, 2017
Received: July 12, 2017

Dear Bill Kurani:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and Part 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely,

Reena Philip -S

Reena Philip, Ph.D.
Director
Division of Molecular Genetics and Pathology
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K163367

Device Name
GenetiSure Dx Postnatal Assay

Indications for Use *(Describe)*

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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5. 510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92(c).

A. 510(k) Number:

K163367

B. Purpose for Submission:

Clearance of new device

C. Submitter Information:

Submitter: Agilent Technologies, Incorporated
5301 Stevens Creek Boulevard
Santa Clara, CA 95051

Establishment Registration No: 2916205

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Date Prepared: November 28, 2016

D. Name of Device and Classification

Name: **GenetiSure Dx Postnatal Assay**

Classification: Class II, 21 CFR § 866.5920 Postnatal chromosomal copy number variation detection system

Product Code: PFX

Panel: Immunology (82)

E. Predicate Device

Affymetrix CytoScan Dx Assay (K130313)

The Agilent GenetiSure Dx Postnatal Assay (the device) is substantially equivalent to the Affymetrix CytoScan Dx Assay (the predicate) as described in the premarket notification K130313.

F. Type of Test or Tests performed:

Chromosomal microarray

G. System Description:

1. Device Description

a) Overview

The GenetiSure Dx Postnatal Assay is a clinical laboratory *in vitro* diagnostic assay for performing molecular karyotyping based on array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) analysis from blood samples of post-natal patients who are suspected of having a genomic abnormality. This molecular karyotyping is a modified *in situ* hybridization technique that allows detection and mapping of DNA sequence copy difference(s) between two genomes in a single experiment. In molecular karyotyping analysis, two differentially labeled genomic DNAs (subject/test sample and a reference sample) are co-hybridized to complementary nucleic acid sequences synthesized *in situ* on a microarray slide.

Locations of copy number variation (CNVs) and copy-neutral loss of heterozygosity (cnLOH) in the DNA segments of the subject sample genome are revealed by variable fluorescence intensity on the microarray.

The assay compares the patient sample against a sex-matched reference sample. Genomic DNA (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.

b) Components of the Product

i. Microarray

K1201A	***GenetiSure Dx Postnatal Assay*** ***K1201-64500 GenetiSure Dx Postnatal Array*** ***K1201-64600 GenetiSure Dx Postnatal Gasket***

Agilent will provide to customers 4x180K aCGH+SNP microarray slides, manufactured under QSR, as the microarray component of this kit. Each single-use microarray slide carries four identical microarrays.

The microarray contains approximately 107,000 probes optimized for CNV analysis, and approximately 59,000 bi-allelic SNP probes. Each probe is approximately 60 bases long.

The CNV probes are distributed across the entire genome with a higher density of probes in regions designated by the International Standards for Cytogenomic Arrays (ISCA) consortium to be of clinical interest. These regions include:

- telomere and selected centromere regions,
- microdeletion / duplication regions,
- dosage sensitive regions, and
- regions associated with X-linked disorders.

Overall, 94% of the genome is targeted with at least 5 CNV probes per 400 kb. The ISCA regions are targeted with a median probe spacing of approximately 1 CNV probes per 3.5 kb.

The SNP probes are designed to known SNPs that overlap restriction digestion sites (Alu I/Rsa I). These probes allow for identification of cnLOH.

Overall, 91% of the genome is targeted with at least 100 SNP probes per 10 Mb.

ii. Gasket slides

The gasket slides are single-use, silicone gasket backing slides that hold the samples during hybridization to the microarrays.

iii. Reagent

K1201-64100 GenetiSure Dx DNA Labeling Kit
K1201-64105 GenetiSure Dx Labeling Kit, -20C Part
K1201-64110 GenetiSure Dx Labeling Kit, RT Components
K1201-64200 GenetiSure Dx Hybridization Kit
K1201-64300 GenetiSure Dx Wash Buffer Set
K1201-64305 GenetiSure Dx Wash Buffer 1, 4L
K1201-64310 GenetiSure Dx Wash Buffer 2, 4L
K1201-64400 GenetiSure Dx Cot-1 Human DNA

A complete list of laboratory equipment and reagents required is provided in the GenetiSure Dx Postnatal Assay Instructions for Use (IFU).

iv. Software

K1203-10000 CytoDx 1.0 Software

Agilent CytoDx Software performs feature extraction, CNV and cnLOH identification and reporting on the microarray TIF images generated by the SureScan Dx Microarray Scanner.

The software application has three functional components, described briefly below.

1) Feature Extraction uses image files created by the scanner, verifies the quality of the scanned image, extracts the intensity information and calculates signal and background for each feature/probe on the array, applying appropriate normalization.

2) CNV and Allele Identifier uses the data produced during the Feature Extraction step to evaluate the intensity of each probe, applying algorithms that identify aberrations, SNPs, CNVs and cnLOH compared to the reference sample.

3) Chromosome Viewer uses the CNV Table and LOH Intervals Table produced during CNV and Allele Identification to overlay the chromosomal aberrations or LOH detected onto an image of the chromosomes to provide a graphical representation of the results.

c) Components Required but Not Included in the System

i. Special Instrument Requirements

G5761A SureScan Dx Microarray Scanner

d) Specimen Processing

The Agilent GenetiSure Dx Postnatal Kit Instructions for Use (IFU) provides instructions to enable the user to process DNA obtained from blood specimens. The illustration and text below provide an overview.

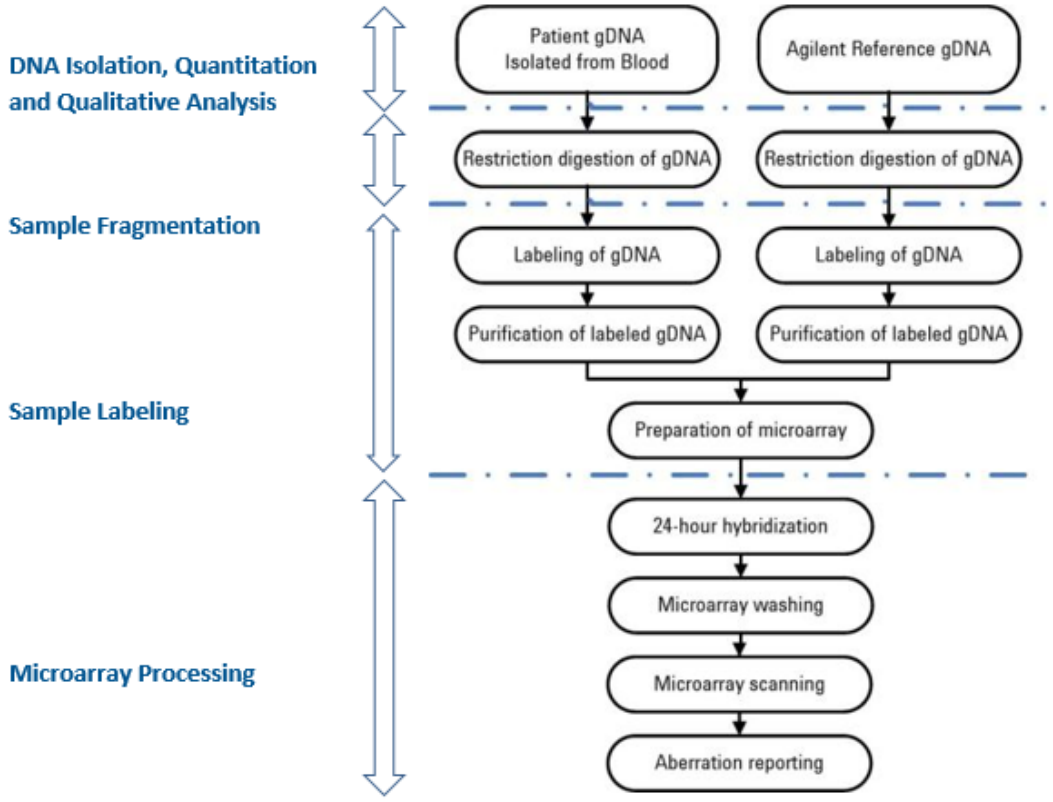

Figure 1-Agilent GenetiSure Dx Postnatal Kit Sample Processing Workflow from gDNA to Report Generation

i. Specimen requirements

The GenetiSure Dx Postnatal Assay is for use with gDNA from whole blood specimens collected in tubes using EDTA as the anticoagulant. Blood specimens may be stored at 2–8°C for up to 7 days prior to DNA extraction. Two hundred (200) microliters of whole blood are used for DNA extraction, and 0.5 micrograms (500 ng) of DNA are required to perform the GenetiSure Dx Postnatal Assay.

ii. Workflow sequence, from sample to report

The Agilent GenetiSure Dx Postnatal Kit starts with purified gDNA isolated from EDTA whole blood samples using the Qiagen QIAmp DSP DNA Blood Mini Kit (part number 61104).

1) gDNA is quantified using a double-stranded DNA-based fluorometric method. For each sample to be tested, 0.5 µg of the subject's DNA is processed in parallel with 0.5 µg of the sex-matched reference DNA included in the GenetiSure Dx DNA Labeling Kit.
2) Both the gDNA of the subject and the sex-matched reference are restriction digested with Alu I and Rsa I restriction enzymes included in the Labeling Kit.
3) After digestion, the samples are labeled in parallel with the reference sample using the fluorescent dyes provided in the Labeling Kit. The subject sample is labeled with cyanine 5 (Cy5) dye and the sex-matched reference sample is labeled with cyanine 3 (Cy3) dye.
4) The two labeled samples are hybridized onto a single microarray of a 4x180k aCGH+SNP slide using the reagents in the GenetiSure Dx Hybridization Kit, GenetiSure Dx Gasket 4xArray Slides (part of the Postnatal Assay) and Hybridization Chamber Kit. Prepared slides are hybridized for 24 hours at 67ºC in a light eliminating Hybridization Oven rotating at 20 rpm.
5) After hybridization, the microarrays are washed using the GenetiSure Dx Wash Buffer Set and transferred into a SureScan Microarray Scanner slide holder.
6) The microarray slides are then scanned in the SureScan Dx Microarray Scanner.
7) The scanner generated image is then processed using the Agilent CytoDx Software, and the image data are converted to numeric data using the Feature Extraction module of the software. The relative abundance of the target sequences is computed by the Analytics module, based on the relative intensities of the fluorophores in the patient and reference samples hybridized to each of the probe sequences. CNVs and cnLOH are reported by chromosomal location.
8) The reported CNVs and cnLOH 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.

iii. Quality Control (QC)

Internal control probes on each array are used to calculate array QC metrics and assess the quality of data. As external controls, Agilent Male and Agilent Female reference DNA (provided with the Labeling Kit) are sex-matched, processed alongside, and co-hybridized with each test sample. These reference DNAs are used for data normalization and aberration detection on a per test sample basis, and to aid in troubleshooting, if necessary.

The following QC checks are required to assure reliable results:

1) Sample input: only samples with sufficient amount of gDNA obtained by DNA extraction/purification procedures proceed to labeling: minimum of 500 ng is required.
2) Labeling/In-Process QC: only samples passing DNA yield and specific activity measurements proceed to array hybridization. The required amount of fluorescently labeled DNA obtained after labeling/purification procedures is 8-15 µg, and the specific activity, i.e. the amount of dye (Cy3 or Cy5) incorporated into DNA after labeling and purification is 20-45 pmol Cy3 dye/µg of DNA or 20-40 pmol Cy5 dye/µg of DNA.
3) Array QC metrics: The software uses the signal from probes on the microarray to perform a series of data verifications that detect laboratory processing anomalies. These include automated grid finding, probe-to-probe noise, signal-to-noise ratios and SNP call rates. Only arrays passing the QC metrics proceed to analysis. If the assay fails any of the array QC metrics, the software will generate a report for review of the QC metrics, but "sign-off" will not be allowed for the report.

2. Description of Test Report

The aberrations identified in a patient sample by the CytoDx algorithms can be viewed from the Triage View screen of the CytoDx software. The final Cyto Report is generated when the Lab Director signs off on the sample results, and lists the aberrations.

Clinical interpretation is performed by a Board Certified Cytogeneticist, Molecular Geneticist, Molecular Pathologist, or similarly qualified clinician who has been trained to identify the clinically relevant CNVs and cnLOH intervals, determine clinical significance, and report out these findings. Clinical interpretation of the aberration results takes place during classification of the aberrations (e.g., as Pathogenic, Likely pathogenic, VOUS, Likely benign, or Benign) in the CytoDx software. To select the appropriate classification, the clinician can rely on prior knowledge, comparisons to tracks or to other samples, and references to gene ontology database that describe the functions and disease associations for genes impacted by the aberrations. The classification assignments appear in the Cyto Report in the Classifications section.

Details of the report are available in the IFU.

H. Intended Use:

1. 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.

2. Indications for Use:

Same as above.

3. Special Condition for Use Statement(s):

For prescription use only.

I. Standard/Guidance Document Referenced (if applicable):

Table 1 – Documents referenced

No	Standard Developing Organization	Standard Designation Number and Date	Title of Standard
1	ISO	14971 Second edition 2007-03-01;14971:2012	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	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
4	AAMI ANSI IEC	62304:2006	Medical device software - Software life cycle processes
5	AAMI ANSI IEC	62366-1 Edition 1.0 2015-02	Medical devices - Part 1: Application of usability engineering to medical devices
6	CLSI	EP07-A2 - 05/21/2007	Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
7	CLSI	EP12-A2 - 01/30/2014	User Protocol for Evaluation of Qualitative Test Performance
8	CLSI	EP25-A - 01/15/2013	Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
9	CLSI	MM13-A - 12/01/2005	Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline
10	CLSI	MM21: 1st Edition – Aug 2015	Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications

J. Substantial Equivalence Discussion

The predicate device for the GenetiSure Dx Postnatal Assay is the Affymetrix CytoScan Dx Assay, cleared in January, 2014 under K130313.

Comparisons between the GenetiSure Dx Postnatal Assay and its predicate device are presented in the following tables.

Table 2-Similarities between assay and predicate

	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, 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.	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.
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.

Report	The device reports the copy number change (gain, loss) 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 or prenatal testing or screening, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.	Same.
Clinical Validation	Compare test results with available diagnosis for sample.	Same.

Table 3-Differences between assay and predicate

	GenetiSure Dx Postnatal Assay (Device)	**Affymetrix CytoScan Dx Assay (Predicate)**
Array Format	60-mer probes Four microarrays on a single 1"x 3" glass slide	25-mer oligos Individual microarrays housed in a GeneChip cartridge
Method of Array Manufacture	On slide (in-situ) synthesis of probes using ink-jet printing	On-wafer synthesis of probes using photolithography
DNA Fragmentation/ Labeling	Fragmented DNA is directly labelled with fluorescent dye (Cy3 and Cy5) before hybridization. Data is produced in two intensity channels which are then compared to generate a LogRatio	Fragmented DNA is PCR amplified and then labelled with biotin before hybridization. Single channel data is produced which is later compared to an in silico reference to produce a LogRatio.
Hybridization	Cohybridization of labeled sample and reference for direct on-array comparison	Hybridization of single labeled sample which is compared to an in silico reference.
Washing / Staining of Microarrays	Manual washing process in accordance with instruction for the validated diagnostic assay	Automated processing with FS450Dx fluidics station for both washing and staining steps
Equipment	SureScan Dx Microarray Scanner	GeneChip System 3000Dx Scanner

K. Performance Characteristics

1. Analytical Performance

a) Reproducibility

The aim of the Reproducibility Study was to demonstrate that GenetiSure Dx Postnatal Assay 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 (copy number gains, losses, 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 (see Table 4). 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 CN gains and losses was 85.0%. Pairwise replicate agreement across the various kb bins ranged from 75.9% to 100%. For copy number gains, the overall agreement was 85.7%; for losses, the overall agreement was 84.6%. For cnLOH, the overall pairwise replicate agreement was 89.1%. Applying a more stringent 80% overlap criteria produced overall agreements of 82.3% for CN gains and losses combined, 84.4% for gains, 81.3% for losses, and 87.9% for cnLOH.

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 cnLOH using the 50% overlap criteria.

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

Table 4-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%
CN Gain	**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
CN Loss	**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 (Gain & Loss)	**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 (see Table 5): 86.2% for combined CN gains and losses

(ranging from 70.6% to 100%), 86.1% for gains alone, 86.3% for losses alone, and 89.1% for cnLOH.

Using the 80% overlap criteria, overall agreements were 84.6% for CN gains and losses combined, 85.3% for gains, 84.2% for losses, and 88.4% for cnLOH. 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 5-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%
CN Gain	**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
CN Loss	**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 (Gain & Loss)	**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.

b) Precision

i. Between-Lot Reagent and Scanner Precision

The aim of the Between-Lot Reagent and Scanner Precision Study was to demonstrate that GenetiSure Dx Postnatal Assay achieves acceptable, precise results when performed using multiple reagent manufacturing lots and when analyzed on multiple scanner instruments. Forty-eight (48) test samples containing a range of chromosomal aberrations (copy number gains, losses, and copy-neutral loss of heterozygosity (cnLOH)) were processed by multiple operators, using combinations of three (3) reagent lots and three (3) scanner instruments across three (3) processing weeks at a single site for a total of 432 data points.

Individual aberrations called within each processed test sample were compared to their respective replicates (9 replicates for each aberration, representing 3x3 reagent-lot/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 83.33%, 98.39%, and 80.80%, respectively. Results were similar when using a more stringent 80% overlap criteria for pairwise replicate agreement. In addition, no substantial differences were observed when the pairwise replicate agreement was assessed separately for inter-lot vs. intra-lot replicate pairs, or for inter-scanner vs. intra-scanner replicate pairs.

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 (see Table 6). 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 copy number gains and losses was 89.0%. Pairwise

replicate agreement across the various kb bins ranged from 76.9% to 100%. For copy number gains, the overall agreement was 85.4%; for copy number losses, the overall agreement was 91.3%. For cnLOH, the overall pairwise replicate agreement was 80.8%. Applying a more stringent 80% overlap criteria produced overall agreements of 87.2% for all CNVs (gains or losses) combined, 84.0% for CN gains, 89.2% for CN losses, and 76.0% for cnLOH.

When assessing specifically the agreement between replicates for positive aberration calls by PPA analysis, the agreement was 93.0% for all copy number calls and 87.3% for cnLOH using the 50% overlap criteria.

Call rate averaged 83.0% for CNVs, and 75.6% for cnLOH.

Table 6-Reproducibility of Aberrations Categorized by Size (in kb) and Type

				Pairwise Replicate Agreement (%)		**PPA (%)**	
Aberration Type	**Aberration Range (kb)**	**# Aberrations**	**Call Rate (%)**	**Overlap**			
				50%	**80%**	**50%**	**80%**
CN Gain	**20-50**	6	59.3	76.9	76.9	80.5	80.5
	50 - 100	6	90.8	85.2	75.5	91.8	81.1
	100 - 200	16	60.3	78.8	77.4	82.5	80.2
	200 - 500	30	62.2	81.9	80.3	85.1	82.4
	500 - 1000	6	61.0	77.8	76.9	78.8	77.3
	1000 - 2000	4	58.3	84.7	84.7	86.9	86.9
	2000 - 5000	18	93.9	88.6	88.6	93.4	93.4
	5000 +	17	100.0	100.0	100.0	100.0	100.0
	Total	103	75.0	85.4	84.0	89.9	88.1
CN Loss	**10-50**	20	70.6	80.3	78.8	86.0	83.9
	50 - 100	4	94.5	90.3	90.3	94.9	94.9
	100 - 200	33	82.8	91.9	89.5	93.8	90.9
	200 - 500	24	81.5	86.8	86.5	91.9	91.5
	500 - 1000	31	92.2	89.5	83.5	93.3	86.8
	1000 - 2000	15	91.9	95.0	93.7	97.2	95.8
	2000 - 5000	6	100.0	100.0	96.3	100.0	96.3
	5000 +	33	99.7	99.3	99.0	99.7	99.3
	Total	166	88.0	91.3	89.2	94.6	92.2
All CNVs	**Total**	269	83.0	89.0	87.2	93.0	90.8

Aberration Type	Aberration Range (kb)	# Aberrations	Call Rate (%)	Pairwise Replicate Agreement (%)		PPA (%)	
				Overlap			
				50%	80%	50%	80%
cnLOH	5000 - 10000	23	66.7	72.7	70.3	79.5	75.9
	10000 - 20000	18	81.5	88.0	78.9	92.6	81.4
	20000 +	5	95.6	92.2	92.2	95.9	95.9
	Total	46	75.6	80.8	76.0	87.3	81.0

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 (see Table 7): 89.6% for combined CN gains and losses (ranging from 70.6% to 100%), 85.6% for CN gains alone, 92.1% for CN losses alone, and 80.8% for cnLOH.

Using the 80% overlap criteria, overall agreements were 88.4% for CN gains and losses combined, 84.6% for CN gains, 90.8% for CN losses, and 76.4% for cnLOH. PPA for the 50% overlap criteria was 93.7% and 87.3% for CNVs and cnLOH, respectively. Call rate averaged 83.0% for CNVs, and 75.6% for cnLOH.

Table 7-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%
CN Gain	5-7	22	51.4	74.7	74.7	75.5	75.5
	7-10	10	51.1	70.6	69.2	71.2	68.5
	10-15	20	76.7	87.8	83.8	91.7	86.4
	15 - 20	14	80.2	82.1	82.1	88.9	88.9
	20 - 30	6	83.3	84.3	83.3	88.9	87.8
	30 - 100	7	74.6	93.7	93.7	95.7	95.7
	100 - 500	10	100.0	100.0	100.0	100.0	100.0
	500 +	14	100.0	100.0	100.0	100.0	100.0
	Total	103	75.0	85.6	84.6	90.2	88.9
CN Loss	5-7	39	72.6	87.6	86.8	91.5	90.4
	7-10	28	81.0	81.9	79.1	88.8	85.3

Aberration Type	Aberration Range (# Probes)	# Aberrations	Call Rate (%)	Pairwise Replicate Agreement (%)		PPA (%)	
				Overlap			
				50%	80%	50%	80%
	10-15	22	87.9	89.8	87.6	94.1	91.7
	15 - 20	12	89.8	94.0	91.0	96.6	93.3
	20 - 30	17	100.0	100.0	100.0	100.0	100.0
	30 - 100	13	99.2	98.3	96.4	99.1	97.2
	100 - 500	15	100.0	100.0	100.0	100.0	100.0
	500 +	20	100.0	100.0	100.0	100.0	100.0
	Total	166	88.0	92.1	90.8	95.5	94.0
All CNVs	**Total**	269	83.0	89.6	88.4	93.7	92.2
cnLOH	**100 to 200**	33	69.4	75.1	72.2	82.0	77.9
	200 to 500	11	89.9	94.4	84.8	96.9	86.2
	500 +	2	100.0	100.0	100.0	100.0	100.0
	Total	46	75.6	80.8	76.4	87.3	81.5

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 CNV (5-20 probes and >20 probes) and cnLOH categories by pairwise confirmation.

ii. 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 Table 8 (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 8 - 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	Gain	36	4326	5391	80.24	NA
5 to 20	Loss	46	5817	6953	83.66	NA
5 to 20	Gain or Loss	82	10143	12344	82.17	70
>20	Gain	29	4244	4387	96.74	NA
>20	Loss	33	4932	4932	100.00	NA
>20	Gain or Loss	62	9176	9319	98.47	80
--	cnLOH	16	1959	2414	81.15	NA

2. 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 selected to maximize the variation across the genome with consideration for gain and loss segments of various sizes/number of probes, chromosomal representation, CNV regions in genic and non-genic regions, and in telomeric and centromeric regions. A total of 2187 CNV regions and 292 cnLOH regions covered 91% of the genome. These aberrations were more prevalent in non-telomeric/non-centromeric regions than in telomeric/centromeric (1337 regions vs 1130 regions). A total of 23% (508 out of 2187 regions) of the CNVs had high (>45%) GC content.

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 9 (All Samples)**.** These statistics remained almost unchanged if all the aberrant samples were considered together (532 samples consisting of both clinical and cell line samples). When considering the 24 normal blood-derived samples only, 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 (5-20 probes), with more being CN Loss than CN Gain. Large CNVs (>20 probes) detected in normal blood samples were relatively rare (Mean =0.25, Median=0 per sample).

Table 9: Aberration Counts Per Sample

All Samples Included in Evaluation (N= 556)						
Distribution	**CN Gain**	**CN Loss**	**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	**CN Gain**	**CN Loss**	**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

Due to the diversified sample panel composition and lack of an applicable universal comparator, independent (non-Agilent) commercially available microarray based assays analytically validated for copy number detection were employed to assist accuracy assessment of CNV aberrations and resolve discrepancies. The samples were tested through the GenetiSure Dx Postnatal Assay using standard procedures in a designated Agilent laboratory.

A target Agilent aberration was deemed "confirmed" if a minimum percent overlap was found with comparator aberration call(s) of the same type (gain, loss, or cnLOH). For the data presented below, a 50% overlap was required, with the Agilent aberrations being compared independently to the other platforms. All eligible Agilent aberrations were assessed, one at a time (Figure 2).

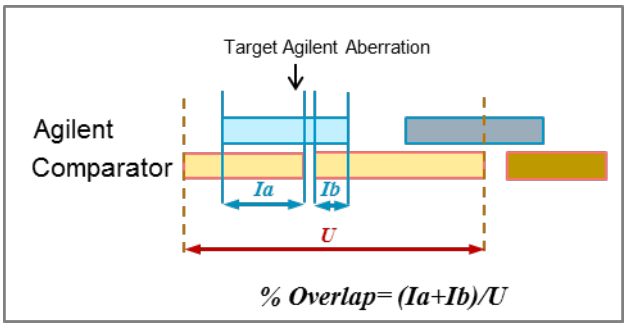

Figure 2: Method for comparison of Agilent detected aberrations with those of comparator platform(s).

If an Agilent CNV aberration could not be "confirmed" by the microarray-based comparator, another analytically validated method (qPCR) was employed to adjudicate the results. To avoid bias in the assessment, an additional 5% randomly selected "confirmed" CNV aberrations (separately selected for CNVs>20 probes and CNVs with 5-20 probes) were also included in this discrepancy resolution testing. Other CNVs directly subject to a third method confirmation included CNVs near the limit of resolution selected from normal whole blood samples.

The results are summarized and stratified by copy number state, size or probe number, and genomic region. The results shown in Table 9 are presented as stratified by probe number, either including indeterminate CNVs as unconfirmed (scheme a) or excluding indeterminate CNVs (scheme b). In Table 10, the results are presented using the same schemes for treatment of indeterminate CNVs, with the results stratified by length in kb.

Table 9: GenetiSure Dx Postnatal Assay Accuracy for all aberration regions Stratified by aberration size (# of probes) when compared to the comparator method (Schemes a & b)

Scheme a: Including Indeterminate CNVs as "Not Confirmed"

TYPE	Aberration Range (# of Probes)	Sample Size (N)*	# Confirmed	Confirmation Rate (95% CI)	FPR (95% CI)
Gain	5-7	48	38	79.2% (65.7%, 88.3%)	20.8% (11.7%, 34.3%)
	7-10	101	85	84.2% (75.8%, 90.0%)	15.8% (10.0%, 24.2%)
	10-15	197	152	77.2% (70.8%, 82.5%)	22.8% (17.5%, 29.2%)
	15-20	101	83	82.2% (73.6%, 88.4%)	17.8% (11.6%, 26.4%)
	20-50	148	104	70.3% (62.5%, 77.0%)	29.7% (23.0%, 37.5%)
	50-500	82	64	78.0% (67.9%, 85.6%)	22.0% (14.4%, 32.1%)
	500 +	169	166	98.2% (94.9%, 99.4%)	1.8% (0.6%, 5.1%)
	Total	846	692	81.8% (79.1%, 84.3%)	18.2% (15.7%, 20.9%)
Loss	5-7	216	196	90.7% (86.1%, 93.9%)	9.3% (6.1%, 13.9%)
	7-10	202	165	81.7% (75.8%, 86.4%)	18.3% (13.6%, 24.2%)
	10-15	257	216	84.0% (79.1%, 88.0%)	16.0% (12.0%, 20.9%)
	15-20	125	90	72.0% (63.6%, 79.1%)	28.0% (20.9%, 36.4%)
	20-50	130	95	73.1% (64.9%, 80.0%)	26.9% (20.0%, 35.1%)
	50-500	225	217	96.4% (93.1%, 98.2%)	3.6% (1.8%, 6.9%)
	500 +	186	180	96.8% (93.1%, 98.5%)	3.2% (1.5%, 6.9%)
	Total	1341	1159	86.4% (84.5%, 88.2%)	13.6% (11.8%, 15.5%)
All CNVs		2187	1851	84.6% (83.1%, 86.1%)	15.4% (13.9%, 16.9%)
cnLOH	100-200	132	94	71.2% (63.0%, 78.2%)	28.8% (21.8%, 37.0%)
	200-500	102	96	94.1% (87.8%, 97.3%)	5.9% (2.7%, 12.2%)

	500 +	58	58	100.0% (93.8%, 100.0%)	0.0% (0.0%, 6.2%)
	Total	292	248	84.9% (80.4%, 88.6%)	15.1% (11.4%, 19.6%)

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

Scheme b: Excluding Indeterminate CNVs

TYPE	Aberration Range (# of Probes)	Sample Size (N)*	# Confirmed	Confirmation Rate (95% CI)	FPR (95% CI)
Gain	5-7	43	38	88.4% (75.5%, 94.9%)	11.6% (5.1%, 24.5%)
	7-10	91	85	93.4% (86.4%, 96.9%)	6.6% (3.1%, 13.6%)
	10-15	175	152	86.9% (81.1%, 91.1%)	13.1% (8.9%, 18.9%)
	15-20	91	83	91.2% (83.6%, 95.5%)	8.8% (4.5%, 16.4%)
	20-50	124	104	83.9% (76.4%, 89.3%)	16.1% (10.7%, 23.6%)
	50-500	72	64	88.9% (79.6%, 94.3%)	11.1% (5.7%, 20.4%)
	500 +	169	166	98.2% (94.9%, 99.4%)	1.8% (0.6%, 5.1%)
	Total	765	692	90.5% (88.2%, 92.3%)	9.5% (7.7%, 11.8%)
Loss	5-7	197	196	99.5% (97.2%, 99.9%)	0.5% (0.1%, 2.8%)
	7-10	183	165	90.2% (85.0%, 93.7%)	9.8% (6.3%, 15.0%)
	10-15	231	216	93.5% (89.6%, 96.0%)	6.5% (4.0%, 10.4%)
	15-20	102	90	88.2% (80.6%, 93.1%)	11.8% (6.9%, 19.4%)
	20-50	114	95	83.3% (75.4%, 89.1%)	16.7% (10.9%, 24.6%)
	50-500	222	217	97.7% (94.8%, 99.0%)	2.3% (1.0%, 5.2%)
	500 +	184	180	97.8% (94.5%, 99.2%)	2.2% (0.8%, 5.5%)
	Total	1233	1159	94.0% (92.5%, 95.2%)	6.0% (4.8%, 7.5%)
All CNVs		1998	1851	92.6% (91.4%, 93.7%)	7.4% (6.3%, 8.6%)
cnLOH	100-200	132	94	71.2% (63.0%, 78.2%)	28.8% (21.8%, 37.0%)
	200-500	99	96	97.0% (91.5%, 99.0%)	3.0% (1.0%, 8.5%)

	500 +	58	58	100.0% (93.8%, 100.0%)	0.0% (0.0%, 6.2%)
	Total	289	248	85.8% (81.3%, 89.4%)	14.2% (10.6%, 18.7%)

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

Table 10: ***GenetiSure Dx Postnatal Assay Accuracy for all aberration regions stratified by aberration size (kb) when compared to the comparator method (Schemes a & b)***

Scheme a: Including Indeterminate CNVs as "Not Confirmed"

TYPE	Aberration Range (kb)	Sample Size (N)*	# Confirmed	Confirmation Rate (95% CI)	FPR (95% CI)
Gain	20-100	69	37	53.6% (42.0%, 64.9%)	46.4% (35.1%, 58.0%)
	100-200	94	75	79.8% (70.6%, 86.7%)	20.2% (13.3%, 29.4%)
	200-300	136	120	88.2% (81.7%, 92.6%)	11.8% (7.4%, 18.3%)
	300-500	123	92	74.8% (66.5%, 81.6%)	25.2% (18.4%, 33.5%)
	500-1,000	90	70	77.8% (68.2%, 85.1%)	22.2% (14.9%, 31.8%)
	1000-10,000	168	136	81.0% (74.3%, 86.2%)	19.0% (13.8%, 25.7%)
	10,000 +	166	162	97.6% (94.0%, 99.1%)	2.4% (0.9%, 6.0%)
	Total	846	692	81.8% (79.1%, 84.3%)	18.2% (15.7%, 20.9%)
Loss	10-100	88	59	67.0% (56.7%, 76.0%)	33.0% (24.0%, 43.3%)
	100-200	207	180	87.0% (81.7%, 90.9%)	13.0% (9.1%, 18.3%)
	200-300	129	114	88.4% (81.7%, 92.8%)	11.6% (7.2%, 18.3%)
	300-500	116	103	88.8% (81.8%, 93.3%)	11.2% (6.7%, 18.2%)
	500-1,000	209	164	78.5% (72.4%, 83.5%)	21.5% (16.5%, 27.6%)

	1000-10,000	398	351	88.2% (84.6%, 91.0%)	11.8% (9.0%, 15.4%)
	10,000 +	194	188	96.9% (93.4%, 98.6%)	3.1% (1.4%, 6.6%)
	Total	1341	1159	86.4% (84.5%, 88.2%)	13.6% (11.8%, 15.5%)
All CNVs		2187	1851	84.6% (83.1%, 86.1%)	15.4% (13.9%, 16.9%)
cnLOH	5,000-10,000	93	61	65.6% (55.5%, 74.5%)	34.4% (25.5%, 44.5%)
	10,000-20,000	94	84	89.4% (81.5%, 94.1%)	10.6% (5.9%, 18.5%)
	20,000 +	105	103	98.1% (93.3%, 99.5%)	1.9% (0.5%, 6.7%)
	Total	292	248	84.9% (80.4%, 88.6%)	15.1% (11.4%, 19.6%)

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

Scheme b: Excluding Indeterminate CNVs

TYPE	Aberration Range (kb)	Sample Size (N)*	# Confirmed	Confirmation Rate (95% CI)	FPR (95% CI)
Gain	20-100	53	37	69.8% (56.5%, 80.5%)	30.2% (19.5%, 43.5%)
	100-200	87	75	86.2% (77.4%, 91.9%)	13.8% (8.1%, 22.6%)
	200-300	123	120	97.6% (93.1%, 99.2%)	2.4% (0.8%, 6.9%)
	300-500	107	92	86.0% (78.2%, 91.3%)	14.0% (8.7%, 21.8%)
	500-1,000	77	70	90.9% (82.4%, 95.5%)	9.1% (4.5%, 17.6%)
	1000-10,000	153	136	88.9% (82.9%, 92.9%)	11.1% (7.1%, 17.1%)
	10,000 +	165	162	98.2% (94.8%, 99.4%)	1.8% (0.6%, 5.2%)

	Total	765	692	90.5% (88.2%, 92.3%)	9.5% (7.7%, 11.8%)
Loss	10-100	76	59	77.6% (67.1%, 85.5%)	22.4% (14.5%, 32.9%)
	100-200	187	180	96.3% (92.5%, 98.2%)	3.7% (1.8%, 7.5%)
	200-300	115	114	99.1% (95.2%, 99.8%)	0.9% (0.2%, 4.8%)
	300-500	105	103	98.1% (93.3%, 99.5%)	1.9% (0.5%, 6.7%)
	500-1,000	179	164	91.6% (86.6%, 94.9%)	8.4% (5.1%, 13.4%)
	1000-10,000	379	351	92.6% (89.5%, 94.8%)	7.4% (5.2%, 10.5%)
	10,000 +	192	188	97.9% (94.8%, 99.2%)	2.1% (0.8%, 5.2%)
	Total	1233	1159	94.0% (92.5%, 95.2%)	6.0% (4.8%, 7.5%)
All CNVs		1998	1851	92.6% (91.4%, 93.7%)	7.4% (6.3%, 8.6%)
cnLOH	5,000-10,000	93	61	65.6% (55.5%, 74.5%)	34.4% (25.5%, 44.5%)
	10,000-20,000	92	84	91.3% (83.8%, 95.5%)	8.7% (4.5%, 16.2%)
	20,000 +	104	103	99.0% (94.8%, 99.8%)	1.0% (0.2%, 5.2%)
	Total	289	248	85.8% (81.3%, 89.4%)	14.2% (10.6%, 18.7%)

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

Analysis was also conducted using alternative methods of comparing the aberrations to the comparator calls. Results using those methods were similar to those presented (data not shown). When calculated using one of the alternative methods, which included a predefined 65% minimum overlap criterion and a composite of aberrations from two platforms as a comparator, and a definitive scheme where by “indeterminate” calls were excluded, the aberration confirmation rates (% agreement with comparator) 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.

The endpoint agreements were also analyzed. Agreements for one comparator are shown in Table 11. Analysis by other methods were found to have similar results.

Table 11: Endpoint Agreement for comparator platform 1; Binning by Number of Probes; Start/Stop Breakpoints Combined; Endpoint Agreement Criteria: ≤2 probes for CNVs (Gain/Loss), ≤50 probes for cnLOH

TYPE	Aberration Range (# of Probes)	Breakpoints, N	Breakpoint Agreement, N	Breakpoint Agreement, % (95% CI)
Gain	5-7	48	47	97.9% (89.1%, 99.6%)
	7-10	126	116	92.1% (86.0%, 95.6%)
	10-15	258	223	86.4% (81.7%, 90.1%)
	15-20	110	88	80.0% (71.6%, 86.4%)
	20-50	128	115	89.8% (83.4%, 94.0%)
	50-500	124	104	83.9% (76.4%, 89.3%)
	500 +	296	258	87.2% (82.9%, 90.5%)
	Total	1090	951	87.2% (85.1%, 89.1%)
Loss	5-7	236	234	99.2% (97.0%, 99.8%)
	7-10	124	117	94.4% (88.8%, 97.2%)
	10-15	116	89	76.7% (68.3%, 83.5%)
	15-20	84	69	82.1% (72.6%, 88.9%)
	20-50	138	110	79.7% (72.2%, 85.6%)
	50-500	420	382	91.0% (87.8%, 93.3%)
	500 +	350	283	80.9% (76.4%, 84.6%)
	Total	1468	1284	87.5% (85.7%, 89.1%)
All CNVs		2558	2235	87.4% (86.0%, 88.6%)
cnLOH	100-200	188	176	93.6% (89.2%, 96.3%)
	200-500	194	176	90.7% (85.8%, 94.1%)
	500 +	116	104	89.7% (82.8%, 94.0%)
	Total	498	456	91.6% (88.8%, 93.7%)

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.

3. Stability

a) Array and Reagent Stability

This study was designed to determine the stability of eight components in the context of the assay workflow.

Stability testing consisted of four different arms of the study:

- Shelf Life: testing after storage of assay components under recommended conditions at defined intervals after manufacturing.
- Multi-use testing: testing after components of the assay that are stored at -15°C to -25°C are subject to different numbers of freeze/thaw cycles
- Transport: Similar to Shelf life testing, but assay components were subject to a transportation simulation prior to storage at recommended conditions.
- In-use testing: testing after different components of the assay are stored for various times after preparation or testing where reaction intermediates are stored for various times.

Results of the study to date indicate that all tested conditions pass all metrics under all conditions tested, supporting a shelf life of the assay components of 12 months. In addition, multi-use and in-use testing support the tested conditions of the assay:

Up to:

- 8x freeze/thaw for GenetiSure Dx DNA Labeling Kit components;
- 16x freeze/thaw or 120 days' storage at -15°C to -25°C for reconstituted 10x Blocking Agent;
- 16x freeze/thaw for Cot-1 DNA;
- 60 days' storage of an open microarray package under specified conditions, and
- 30 days' storage of the digested gDNA or labeled DNA reaction intermediates at -15°C to -25°C.

b) Whole Blood Stability

To determine the stability of whole blood specimens prior to gDNA isolation, 24 whole blood specimens, 12 male and 12 female, were obtained from a blood bank and gDNA was isolated from the specimens at 1, 3, 7, and 10 days after initial collection.

A list of aberrations for each sample extracted on Days 3, 7 and 10 were reported and compared with the 'Day 1' list for the same sample. An aberration from the 'Day 1' sample was considered confirmed in the stored samples if the test result identifies a region of aberration that overlaps the 'Day 1' region by at least 50%. Blood was considered stable when stored for a given time when 75% of small CNV (gain/loss) aberrations, called by 5-20 copy number probes, and 90% of the larger CNV (gain/loss)

aberrations, called by >20 probes, were confirmed. An additional analysis was also performed using an 80% overlap criteria.

The results of both the 50% and 80% overlap analysis methods demonstrated that whole blood specimens may be stored for up to 10 days at 2–8ºC prior to gDNA isolation. Samples stored for this period of time and processed with the GenetiSure Dx Postnatal Assay produced acceptable results.

4. Limit of Detection

a) DNA Input

To determine the analytical sensitivity, or the Limit of Detection (LOD) of the GenetiSure Dx Postnatal Assay, 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.

b) Mosaicism

To determine the sensitivity (LoD) 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 gains and losses. Smaller aberrations could not be reliability 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 copy number gains and losses (>100 probes) down to the 50% level.

5. Analytical Specificity

a) 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 CNVs (gains/losses) in the 5-20 probe category and 90% of the CNVs (gains/losses) 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.

b) 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.

6. Clinical Validity

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), gains and losses, 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. 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.

a) 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 12: 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 (Table 13) or considering both

copy number and cnLOH aberrations (Table 14). 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 13: 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 Interpretation	Pathogenic	56	14	9	0	3	82
	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]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. 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 14: 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]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.

A list of observed syndromes was compiled based on the pathogenic and likely pathogenic calls detected from the 800 SPA samples (Table 15). In total, 36 distinct syndromes were identified which encompassed 73 cases from the clinical study sample set.

Table 15: List of Syndromes in the Clinical Study Sample Set

Syndrome Association	Number of Cases
10q26 Deletion Syndrome	1
13q Deletion Syndrome	1
15q11.2 Deletion Syndrome	5
15q13.3 Microdeletion Syndrome	1

Syndrome Association	Number of Cases
15q25 Deletion Syndrome	1
16p11.2 Microdeletion	1
16p11.2 Microduplication	2
16p12.1 Deletion Syndrome	1
16p13.11 Microdeletion	2
16p13.11 Microduplication neurocognitive disorder susceptibility locus	3
1q21.1 Deletion Syndrome	2
1q21.1 Duplication Syndrome	2
22q11.2 Duplication Syndrome	3
2q37 Deletion Syndrome	4
3q29 Deletion Syndrome	1
7q11.23 Duplication Syndrome	1
8p23.1 Microdeletion/CDH syndrome	1
8p23.1 Microduplication	1
Angelman/Prader-Willi Syndrome	6
Charcot-Marie-Tooth Neuropathy, Type 1a	1
DiGeorge Syndrome	7
Distal 22q11.2 Deletion Syndrome	1
Downs Syndrome/Trisomy 21	6
Ichthyosis, X-Linked/ STS Deficiency	1
Isodicentric Chromosome 15 Syndrome	1
Jacobsen/ 11q Deletion	1
Klinefelter Syndrome	4
Mental Retardation-Hypotonic Facies Syndrome, X-Linked/ Smith-Fineman-Myers	1
Neuropathy, Hereditary, With Liability to Pressure Palsies; HNPP	1
Sotos Syndrome-1/ 5q35 Deletion Syndrome	1
Triple X Syndrome	2
Trisomy 9 mosaicism	1
Turner Syndrome	1

Syndrome Association	Number of Cases
Williams-Beuren Syndrome	2
Xq26.3 Duplication Syndrome	1
Other	2
Total	**73**

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 15-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 in Table 17

.

Table 17-Pathogenic and Likely Pathogenic Aberrations Detected in Phenotypically Normal Samples

Sample ID	Type	Size	Chr Band	Interpretation
CLIS-CMC-0033-F	Gain	100 kb	22q13.33	Pathogenic
CLIS-CMC-0216-F	Loss	18.2 kb	16q24.3	Pathogenic
CLIS-CMC-0253-M	Gain	646 kb	Xp21.2-p21.1	L. Pathogenic
CLIS-CMC-0259-F	cnLOH	20 Mb	2q14.3-q22.3	L. Pathogenic
CLIS-CMC-0281-F	Loss	13.6 kb	15q11.2	L. Pathogenic
CLIS-CMC-0372-M	cnLOH	11 Mb	6q23.3-q25.1	L. Pathogenic
CLIS-CMC-0497-M	Gain	59 Mb+93 Mb	Xp + Xq	Pathogenic
CLIS-CMC-0507-F	Gain	1.5 Mb	16p13.11	L. Pathogenic

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 gain 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 Klinfelter 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.