Cell-Free Fetal DNA Testing (for New Jersey Only)

Policy Number: CS085NJ.Y
Effective Date: September 1, 2024

➲ Instructions for Use

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Related Policy

- Chromosome Microarray Testing (Non-Oncology Conditions) (for New Jersey Only)

Application

This Medical Policy only applies to the state of New Jersey.

Coverage Rationale

DNA-based noninvasive prenatal tests of fetal Aneuploidy are proven and medically necessary as screening tools for Trisomy 21 (Down Syndrome), Trisomy 18 (Edwards Syndrome), or Trisomy 13 (Patau Syndrome), with or without fetal sex chromosomes, for individuals with a singleton or twin pregnancy in any one of the following circumstances:

- Birthing person aged 35 years or older at delivery and/or donor oocyte aged 35 years or older; or
- Fetal ultrasound findings indicating an increased risk of Aneuploidy; or
- History of a prior pregnancy with a trisomy due to translocation; or
- Positive first- or second-trimester screening test results for Aneuploidy; or
- Parental balanced Robertsonian translocation with an increased risk of fetal Trisomy 13 or Trisomy 21; or
- Screening after pre-test counseling from a board-certified genetic counselor or from the prenatal care physician or healthcare professional using Shared Decision-Making (SDM)

Due to insufficient evidence of efficacy, DNA-based noninvasive prenatal tests are unproven and not medically necessary for all other indications, including:

- For the sole purpose of determining the sex of the fetus unless the determination of fetal sex is essential to the diagnosis of a condition
- For the sole purpose of determining twin zygosity
- Repeat testing due to low fetal fraction
- Genome-wide or exome-wide screening (e.g., MaterniT Genome)
- Pregnancies involving one or more of the following:
  - Three or more fetuses
  - Missed abortion or fetal demise in a single or multiple gestation pregnancy
  - Vanishing twin syndrome
- Screening for the following:
  - Aneuploidy other than trisomies 21, 18, 13, or sex chromosomes
  - Microdeletions/microduplications/copy number variations (CNVs)
  - Single gene disorders (e.g., Vistara™, PreSeek™)

- Fetal RhD or fetal antigen status

Due to insufficient evidence of efficacy, the following DNA-based noninvasive prenatal test is unproven and not medically necessary:

- Vanadis®

Genetic Counseling

Genetic counseling is strongly recommended prior to fetal screening or prenatal diagnosis in order to inform persons being tested about the advantages and limitations of the test as applied to a unique person.

Definitions

Aneuploidy: A normal human cell has 23 pairs of chromosomes. An abnormal number of chromosomes in a human cell is called aneuploidy. This includes trisomy, where there is an extra chromosome present, or monosomy, where a chromosome is missing. Aneuploidy can impact any of the chromosomes, including sex chromosomes. Down Syndrome (Trisomy 21) is a common aneuploidy. Patau Syndrome (Trisomy 13) and Edwards Syndrome (Trisomy 18) are other notable aneuploidies [American College of Obstetricians and Gynecologists (ACOG) Dictionary, 2024].

Cell Free Fetal DNA (cffDNA or cfDNA): Small fragments of fetal DNA from the placenta that move freely in the pregnant individual's blood. These fragments can be analyzed via a noninvasive prenatal screening test. (ACOG Dictionary, 2024).

Comparative Genomic Hybridization (CGH): CGH is a technology that can be used for the detection of genomic copy number variations (CNVs). Tests can use a variety of probes or Single Nucleotide Polymorphisms (SNPs) to provide copy number and gene differentiating information. All platforms share in common that individual and reference DNA are labelled with dyes or fluorescing probes and hybridized on the array. A scanner then measures differences in intensity between the probes, and the data is expressed as having greater or less intensity than the reference DNA (South et al., 2013).

Massively Parallel Sequencing (MPS): Also referred to as Next Generation Sequencing (NGS), as well as Massively Parallel Shotgun Sequencing (MPSS), this technology allows for the simultaneous sequencing of multiple genes at the same time on a solid surface like a glass slide or bead (Alekseyev et al., 2018).

Mosaicism: An error in cell division may cause an individual to have two or more different populations of cells that have different chromosomes. One example is mosaic Turner syndrome, where some cells are 46, XX and others are 45, X due to the loss of a chromosome (MedlinePlus, 2022a).

Next Generation Sequencing (NGS): New sequencing techniques that can quickly analyze multiple sections of DNA at the same time. Older forms of sequencing could only analyze one section of DNA at once (Alekseyev et al., 2018).

Non-Invasive Prenatal Testing/Screening (NIPT/NIPS): A common term used to describe different types of analysis of cffDNA (Allyse and Wick, 2018).

Shared Decision-Making (SDM): SDM is a process by which physicians and individuals work together to choose the treatment option that best reflects the clinical evidence and the individual's values and preferences (Armstrong and Metlay, 2020).

Single Nucleotide Polymorphisms (SNPs): Small variations in an individual's DNA occur about once every 1,000 nucleotides. These small differences, SNPs, usually have no impact on health or development but help identify specific chromosomal locations in the DNA (MedlinePlus, 2022b).

Trisomy 13 (Patau Syndrome): A chromosomal condition with an extra chromosome 13. It is associated with multiple congenital anomalies and significant developmental delay. Most infants die in the first month after birth, with only 5-10% surviving past the first year. The risk of having a child with Trisomy 13 increases with a mother's age (MedlinePlus, 2021a).

Trisomy 18 (Edwards Syndrome): A chromosomal condition with an extra chromosome 18. It is associated with multiple congenital anomalies and developmental delay. Most infants die in the first year of life, with only 5-10% surviving past the first year. The risk of having a child with Trisomy 18 increases with a mother's age (MedlinePlus, 2021b).

Trisomy 21 (Down Syndrome): A chromosomal condition with an extra chromosome 21. It is associated with intellectual disability, a characteristic facial appearance and poor muscle tone (hypotonia) in infancy. The degree of intellectual disability varies, but it is usually mild to moderate. Individuals with Down Syndrome may be born with a variety of birth defects, including heart defects and digestive abnormalities. The risk of having a child with Trisomy 21 increases with a mother's age (MedlinePlus, 2020).

Twin Zygosity: Zygosity refers to the type of conception. Dizygotic (nonidentical, fraternal) twins result from multiple ovulations with (near) synchronous fertilization of two separate ova by two separate sperm cells. Dizygotic twins thus share the same genetic relationship as nontwin siblings and share approximately 50% of genes. Monozygotic twins (so-called identical twins) are generated by division of a zygote that originated from the fertilization of one single ovum by one single sperm cell (De Paepe, 2023).

Whole Genome Sequencing (WGS): WGS determines the sequence of the entire DNA in a person, or a tissue type, such as a tumor, which includes the protein making (coding) as well as non-coding DNA elements (MedlinePlus, 2021c).

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by federal, state, or contractual requirements and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
0060U	Twin zygosity, genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood
*0327U	Fetal aneuploidy (trisomy 13, 18, and 21), DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy, includes sex reporting, if performed
81420	Fetal chromosomal aneuploidy (e.g., trisomy 21, monosomy X) genomic sequence analysis panel, circulating cell-free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18, and 21
81422	Fetal chromosomal microdeletion(s) genomic sequence analysis (e.g., DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood
81479	Unlisted molecular pathology procedure
81507	Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy

Codes labeled with an asterisk (*) are not on the State of New Jersey Medicaid Fee Schedule and therefore may not be covered by the State of New Jersey Medicaid Program.

Diagnosis Code	Description
O09.00	Supervision of pregnancy with history of infertility, unspecified trimester
O09.01	Supervision of pregnancy with history of infertility, first trimester
O09.02	Supervision of pregnancy with history of infertility, second trimester
O09.03	Supervision of pregnancy with history of infertility, third trimester
O09.10	Supervision of pregnancy with history of ectopic pregnancy, unspecified trimester
O09.11	Supervision of pregnancy with history of ectopic pregnancy, first trimester
O09.12	Supervision of pregnancy with history of ectopic pregnancy, second trimester
O09.13	Supervision of pregnancy with history of ectopic pregnancy, third trimester
O09.211	Supervision of pregnancy with history of pre-term labor, first trimester
O09.212	Supervision of pregnancy with history of pre-term labor, second trimester
O09.213	Supervision of pregnancy with history of pre-term labor, third trimester

Diagnosis Code	Description
O09.219	Supervision of pregnancy with history of pre-term labor, unspecified trimester
O09.291	Supervision of pregnancy with other poor reproductive or obstetric history, first trimester
O09.292	Supervision of pregnancy with other poor reproductive or obstetric history, second trimester
O09.293	Supervision of pregnancy with other poor reproductive or obstetric history, third trimester
O09.299	Supervision of pregnancy with other poor reproductive or obstetric history, unspecified trimester
O09.30	Supervision of pregnancy with insufficient antenatal care, unspecified trimester
O09.31	Supervision of pregnancy with insufficient antenatal care, first trimester
O09.32	Supervision of pregnancy with insufficient antenatal care, second trimester
O09.33	Supervision of pregnancy with insufficient antenatal care, third trimester
O09.40	Supervision of pregnancy with grand multiparity, unspecified trimester
O09.41	Supervision of pregnancy with grand multiparity, first trimester
O09.42	Supervision of pregnancy with grand multiparity, second trimester
O09.43	Supervision of pregnancy with grand multiparity, third trimester
O09.511	Supervision of elderly primigravida, first trimester
O09.512	Supervision of elderly primigravida, second trimester
O09.513	Supervision of elderly primigravida, third trimester
O09.519	Supervision of elderly primigravida, unspecified trimester
O09.521	Supervision of elderly multigravida, first trimester
O09.522	Supervision of elderly multigravida, second trimester
O09.523	Supervision of elderly multigravida, third trimester
O09.529	Supervision of elderly multigravida, unspecified trimester
O09.611	Supervision of young primigravida, first trimester
O09.612	Supervision of young primigravida, second trimester
O09.613	Supervision of young primigravida, third trimester
O09.619	Supervision of young primigravida, unspecified trimester
O09.621	Supervision of young multigravida, first trimester
O09.622	Supervision of young multigravida, second trimester
O09.623	Supervision of young multigravida, third trimester
O09.629	Supervision of young multigravida, unspecified trimester
O09.70	Supervision of high risk pregnancy due to social problems, unspecified trimester
O09.71	Supervision of high risk pregnancy due to social problems, first trimester
O09.72	Supervision of high risk pregnancy due to social problems, second trimester
O09.73	Supervision of high risk pregnancy due to social problems, third trimester
O09.811	Supervision of pregnancy resulting from assisted reproductive technology, first trimester
O09.812	Supervision of pregnancy resulting from assisted reproductive technology, second trimester
O09.813	Supervision of pregnancy resulting from assisted reproductive technology, third trimester
O09.819	Supervision of pregnancy resulting from assisted reproductive technology, unspecified trimester
O09.821	Supervision of pregnancy with history of in utero procedure during previous pregnancy, first trimester
O09.822	Supervision of pregnancy with history of in utero procedure during previous pregnancy, second trimester
O09.823	Supervision of pregnancy with history of in utero procedure during previous pregnancy, third trimester
O09.829	Supervision of pregnancy with history of in utero procedure during previous pregnancy, unspecified trimester
O09.891	Supervision of other high risk pregnancies, first trimester

Diagnosis Code	Description
O09.892	Supervision of other high risk pregnancies, second trimester
O09.893	Supervision of other high risk pregnancies, third trimester
O09.899	Supervision of other high risk pregnancies, unspecified trimester
O09.90	Supervision of high risk pregnancy, unspecified, unspecified trimester
O09.91	Supervision of high risk pregnancy, unspecified, first trimester
O09.92	Supervision of high risk pregnancy, unspecified, second trimester
O09.93	Supervision of high risk pregnancy, unspecified, third trimester
O09.A0	Supervision of pregnancy with history of molar pregnancy, unspecified trimester
O09.A1	Supervision of pregnancy with history of molar pregnancy, first trimester
O09.A2	Supervision of pregnancy with history of molar pregnancy, second trimester
O09.A3	Supervision of pregnancy with history of molar pregnancy, third trimester
O26.20	Pregnancy care for patient with recurrent pregnancy loss, unspecified trimester
O26.21	Pregnancy care for patient with recurrent pregnancy loss, first trimester
O26.22	Pregnancy care for patient with recurrent pregnancy loss, second trimester
O26.23	Pregnancy care for patient with recurrent pregnancy loss, third trimester
O26.841	Uterine size-date discrepancy, first trimester
O26.842	Uterine size-date discrepancy, second trimester
O26.843	Uterine size-date discrepancy, third trimester
O26.849	Uterine size-date discrepancy, unspecified trimester
O26.851	Spotting complicating pregnancy, first trimester
O26.852	Spotting complicating pregnancy, second trimester
O26.853	Spotting complicating pregnancy, third trimester
O26.859	Spotting complicating pregnancy, unspecified trimester
O26.891	Other specified pregnancy related conditions, first trimester
O26.892	Other specified pregnancy related conditions, second trimester
O26.893	Other specified pregnancy related conditions, third trimester
O26.899	Other specified pregnancy related conditions, unspecified trimester
O26.90	Pregnancy related conditions, unspecified, unspecified trimester
O26.91	Pregnancy related conditions, unspecified, first trimester
O26.92	Pregnancy related conditions, unspecified, second trimester
O26.93	Pregnancy related conditions, unspecified, third trimester
O28.0	Abnormal hematological finding on antenatal screening of mother
O28.1	Abnormal biochemical finding on antenatal screening of mother
O28.2	Abnormal cytological finding on antenatal screening of mother
O28.3	Abnormal ultrasonic finding on antenatal screening of mother
O28.4	Abnormal radiological finding on antenatal screening of mother
O28.5	Abnormal chromosomal and genetic finding on antenatal screening of mother
O28.8	Other abnormal findings on antenatal screening of mother
O28.9	Unspecified abnormal findings on antenatal screening of mother
O30.001	Twin pregnancy, unspecified number of placenta and unspecified number of amniotic sacs, first trimester
O30.002	Twin pregnancy, unspecified number of placenta and unspecified number of amniotic sacs, second trimester
O30.003	Twin pregnancy, unspecified number of placenta and unspecified number of amniotic sacs, third trimester

Diagnosis Code	Description
O30.009	Twin pregnancy, unspecified number of placenta and unspecified number of amniotic sacs, unspecified trimester
O30.011	Twin pregnancy, monochorionic/monoamniotic, first trimester
O30.012	Twin pregnancy, monochorionic/monoamniotic, second trimester
O30.013	Twin pregnancy, monochorionic/monoamniotic, third trimester
O30.019	Twin pregnancy, monochorionic/monoamniotic, unspecified trimester
O30.021	Conjoined twin pregnancy, first trimester
O30.022	Conjoined twin pregnancy, second trimester
O30.023	Conjoined twin pregnancy, third trimester
O30.029	Conjoined twin pregnancy, unspecified trimester
O30.031	Twin pregnancy, monochorionic/diamniotic, first trimester
O30.032	Twin pregnancy, monochorionic/diamniotic, second trimester
O30.033	Twin pregnancy, monochorionic/diamniotic, third trimester
O30.039	Twin pregnancy, monochorionic/diamniotic, unspecified trimester
O30.041	Twin pregnancy, dichorionic/diamniotic, first trimester
O30.042	Twin pregnancy, dichorionic/diamniotic, second trimester
O30.043	Twin pregnancy, dichorionic/diamniotic, third trimester
O30.049	Twin pregnancy, dichorionic/diamniotic, unspecified trimester
O30.091	Twin pregnancy, unable to determine number of placenta and number of amniotic sacs, first trimester
O30.092	Twin pregnancy, unable to determine number of placenta and number of amniotic sacs, second trimester
O30.093	Twin pregnancy, unable to determine number of placenta and number of amniotic sacs, third trimester
O30.099	Twin pregnancy, unable to determine number of placenta and number of amniotic sacs, unspecified trimester
O35.00X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, unspecified, not applicable or unspecified
O35.01X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, agenesis of the corpus callosum, not applicable or unspecified
O35.02X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, anencephaly, not applicable or unspecified
O35.03X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, choroid plexus cysts, not applicable or unspecified
O35.04X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, encephalocele, not applicable or unspecified
O35.05X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, holoprosencephaly, not applicable or unspecified
O35.06X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, hydrocephaly, not applicable or unspecified
O35.07X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, microcephaly, not applicable or unspecified
O35.08X0	Maternal care for (suspected) central nervous system malformation or damage in fetus, spina bifida, not applicable or unspecified
O35.09X0	Maternal care for (suspected) other central nervous system malformation or damage in fetus, not applicable or unspecified
O35.10X0	Maternal care for (suspected) chromosomal abnormality in fetus, unspecified, not applicable or unspecified

Diagnosis Code	Description
O35.11X0	Maternal care for (suspected) chromosomal abnormality in fetus, Trisomy 13, not applicable or unspecified
O35.12X0	Maternal care for (suspected) chromosomal abnormality in fetus, Trisomy 18, not applicable or unspecified
O35.13X0	Maternal care for (suspected) chromosomal abnormality in fetus, Trisomy 21, not applicable or unspecified
O35.14X0	Maternal care for (suspected) chromosomal abnormality in fetus, Turner Syndrome, not applicable or unspecified
O35.15X0	Maternal care for (suspected) chromosomal abnormality in fetus, sex chromosome abnormality, not applicable or unspecified
O35.19X0	Maternal care for (suspected) chromosomal abnormality in fetus, other chromosomal abnormality, not applicable or unspecified
O35.2XX0	Maternal care for (suspected) hereditary disease in fetus, not applicable or unspecified
O35.AXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal facial anomalies, not applicable or unspecified
O35.BXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal cardiac anomalies, not applicable or unspecified
O35.CXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal pulmonary anomalies, not applicable or unspecified
O35.DXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal gastrointestinal anomalies, not applicable or unspecified
O35.EXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal genitourinary anomalies, not applicable or unspecified
O35.FXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal musculoskeletal anomalies of trunk, not applicable or unspecified
O35.GXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal upper extremities anomalies, not applicable or unspecified
O35.HXX0	Maternal care for other (suspected) fetal abnormality and damage, fetal lower extremities anomalies, not applicable or unspecified
O99.210	Obesity complicating pregnancy, unspecified trimester
O99.211	Obesity complicating pregnancy, first trimester
O99.212	Obesity complicating pregnancy, second trimester
O99.213	Obesity complicating pregnancy, third trimester
O99.280	Endocrine, nutritional and metabolic diseases complicating pregnancy, unspecified trimester
O99.281	Endocrine, nutritional and metabolic diseases complicating pregnancy, first trimester
O99.282	Endocrine, nutritional and metabolic diseases complicating pregnancy, second trimester
O99.283	Endocrine, nutritional and metabolic diseases complicating pregnancy, third trimester
O99.284	Endocrine, nutritional and metabolic diseases complicating childbirth
O99.285	Endocrine, nutritional and metabolic diseases complicating the puerperium
O99.310	Alcohol use complicating pregnancy, unspecified trimester
O99.311	Alcohol use complicating pregnancy, first trimester
O99.312	Alcohol use complicating pregnancy, second trimester
O99.313	Alcohol use complicating pregnancy, third trimester
O99.320	Drug use complicating pregnancy, unspecified trimester
O99.321	Drug use complicating pregnancy, first trimester
O99.322	Drug use complicating pregnancy, second trimester
O99.323	Drug use complicating pregnancy, third trimester
O99.330	Smoking (tobacco) complicating pregnancy, unspecified trimester

Diagnosis Code	Description
O99.331	Smoking (tobacco) complicating pregnancy, first trimester
O99.332	Smoking (tobacco) complicating pregnancy, second trimester
O99.333	Smoking (tobacco) complicating pregnancy, third trimester
O99.340	Other mental disorders complicating pregnancy, unspecified trimester
O99.341	Other mental disorders complicating pregnancy, first trimester
O99.342	Other mental disorders complicating pregnancy, second trimester
O99.343	Other mental disorders complicating pregnancy, third trimester
O99.810	Abnormal glucose complicating pregnancy
O99.814	Abnormal glucose complicating childbirth
Q95.0	Balanced translocation and insertion in normal individual
Q95.1	Chromosome inversion in normal individual
Q95.2	Balanced autosomal rearrangement in abnormal individual
Q95.3	Balanced sex/autosomal rearrangement in abnormal individual
Q95.5	Individual with autosomal fragile site
Q95.8	Other balanced rearrangements and structural markers
Q95.9	Balanced rearrangement and structural marker, unspecified
Z34.00	Encounter for supervision of normal first pregnancy, unspecified trimester
Z34.01	Encounter for supervision of normal first pregnancy, first trimester
Z34.02	Encounter for supervision of normal first pregnancy, second trimester
Z34.03	Encounter for supervision of normal first pregnancy, third trimester
Z34.80	Encounter for supervision of other normal pregnancy, unspecified trimester
Z34.81	Encounter for supervision of other normal pregnancy, first trimester
Z34.82	Encounter for supervision of other normal pregnancy, second trimester
Z34.83	Encounter for supervision of other normal pregnancy, third trimester
Z34.90	Encounter for supervision of normal pregnancy, unspecified, unspecified trimester
Z34.91	Encounter for supervision of normal pregnancy, unspecified, first trimester
Z34.92	Encounter for supervision of normal pregnancy, unspecified, second trimester
Z34.93	Encounter for supervision of normal pregnancy, unspecified, third trimester
Z36.0	Encounter for antenatal screening for chromosomal anomalies
Z36.1	Encounter for antenatal screening for raised alphafetoprotein level
Z36.2	Encounter for other antenatal screening follow-up
Z36.3	Encounter for antenatal screening for malformations
Z36.4	Encounter for antenatal screening for fetal growth retardation
Z36.5	Encounter for antenatal screening for isoimmunization
Z36.81	Encounter for antenatal screening for hydrops fetalis
Z36.82	Encounter for antenatal screening for nuchal translucency
Z36.83	Encounter for fetal screening for congenital cardiac abnormalities
Z36.89	Encounter for other specified antenatal screening
Z36.8A	Encounter for antenatal screening for other genetic defects
Z36.9	Encounter for antenatal screening, unspecified
Z3A.09	9 weeks gestation of pregnancy
Z3A.10	10 weeks gestation of pregnancy
Z3A.11	11 weeks gestation of pregnancy
Z3A.12	12 weeks gestation of pregnancy
Z3A.13	13 weeks gestation of pregnancy

Diagnosis Code	Description
Z3A.14	14 weeks gestation of pregnancy
Z3A.15	15 weeks gestation of pregnancy
Z3A.16	16 weeks gestation of pregnancy
Z3A.17	17 weeks gestation of pregnancy
Z3A.18	18 weeks gestation of pregnancy
Z3A.19	19 weeks gestation of pregnancy
Z3A.20	20 weeks gestation of pregnancy
Z3A.21	21 weeks gestation of pregnancy
Z3A.22	22 weeks gestation of pregnancy
Z3A.23	23 weeks gestation of pregnancy
Z3A.24	24 weeks gestation of pregnancy
Z3A.25	25 weeks gestation of pregnancy
Z3A.26	26 weeks gestation of pregnancy
Z3A.27	27 weeks gestation of pregnancy
Z3A.28	28 weeks gestation of pregnancy
Z3A.29	29 weeks gestation of pregnancy
Z3A.30	30 weeks gestation of pregnancy
Z3A.31	31 weeks gestation of pregnancy
Z3A.32	32 weeks gestation of pregnancy
Z3A.33	33 weeks gestation of pregnancy
Z3A.34	34 weeks gestation of pregnancy
Z3A.35	35 weeks gestation of pregnancy
Z3A.36	36 weeks gestation of pregnancy
Z3A.37	37 weeks gestation of pregnancy
Z3A.38	38 weeks gestation of pregnancy
Z3A.39	39 weeks gestation of pregnancy
Z3A.40	40 weeks gestation of pregnancy
Z3A.41	41 weeks gestation of pregnancy
Z3A.42	42 weeks gestation of pregnancy
Z3A.49	Greater than 42 weeks gestation of pregnancy

Description of Services

Historically, screening tests for Trisomy 13, 18, and 21 have included first-trimester screening (which involves an ultrasound and a blood test) and maternal serum screening (a blood test) in addition to a high-resolution ultrasound evaluation in the second trimester. In the past decade, prenatal screening performed using cell-free DNA (cfDNA) has become more common. During pregnancy, cfDNA from the placenta circulates in the birthing person's blood. Fetal cfDNA from this blood can be screened for aneuploidies and other genetic anomalies, with testing offered as early as 10 weeks gestation. Available tests use different methodologies and algorithms for data analysis. These tests may identify women with an increased risk of having a child with Trisomy 13, 18 or 21, but they cannot diagnose, confirm, or exclude the possibility of a chromosomal disorder. Only conventional prenatal diagnosis (i.e., chorionic villus sampling (CVS) or amniocentesis) can definitively diagnose fetal trisomies (ACOG, 2020).

Clinical Evidence

Aneuploidy Screening for Trisomies 13, 18, 21, and Sex Chromosomes

In an effort to evaluate the performance of cell-free DNA (cfDNA) screening for trisomy 21, Dugoff et al. (2023) conducted a retrospective cohort study in a large and diverse group of individuals pregnant with twins. The performance of cfDNA screening for trisomy 13 and 18 was assessed as well. The study initially included 1764 individuals from 17 centers who

were screened with cfDNA testing from December 2011 to February 2020. The method of testing used was massively parallel sequencing and all tests were performed in a single laboratory. The researchers reviewed medical records for all newborns; information regarding birth outcome, presence of congenital abnormalities, phenotypic appearance and results of any chromosomal testing performed in the antenatal or postnatal period were extracted. In circumstances where there was a possibility of fetal chromosomal abnormality and no genetic test results were available, a committee of maternal-fetal medicine genetic experts reviewed the data. Pregnancies that were found to have vanishing twin (n = 78) or cases with inadequate follow up (n = 239) were excluded from the analysis, resulting in a total of 1447 cases included in the study results. Median age of the pregnant individual was 35 years and the median gestational age when cfDNA testing occurred was 12.3 weeks, with median fetal fraction (FF) of 12.4%. CfDNA testing identified a total of 41 of the 42 pregnancies where one or both fetuses were affected by trisomy 21, equating to a detection rate (DR) of 97.6% (95% confidence interval [CI], 83.8-99.7). In cases of trisomy 18, all ten of the ten impacted pregnancies were detected and there was one false positive. For trisomy 13, DR was 80% (4 of 5 true cases was detected). The rate of nonreportable results was low, at 3.9%. Based on this data, the authors concluded that cfDNA testing is an effective method to screen for trisomy 21 beginning in the first trimester of a twin gestation pregnancy. DR was high in both dichorionic and monochorionic twins with low nonreportable rates. The researchers note, however, that differing cfDNA screening methods may lead to different performance across labs. In addition, although the study participants were geographically diverse, the majority of individuals tested were white, and median BMI was 25.1; populations with larger proportions of non-white individuals and/or higher incidence of obesity could impact test performance. Interestingly, this cohort included a high number of cases of trisomy 13 and 18 when compared with current literature reports and while the use of cfDNA screening for these two conditions appears promising, the volume of impacted individuals in this study were too small to reach definitive conclusions. Overall, this study confirms the effectiveness of cfDNA screening for trisomy 21 in twin pregnancy and bolsters the existing evidence related to this screening for trisomy 13 and 18.

To determine the accuracy of cfDNA testing for the detection of SCAs in single gestation pregnancies, Shear et al. (2023) conducted a systematic review and meta-analysis of the published literature. Studies assessed were limited to those published between January 2010 and December 2021 that included results for both cfDNA and confirmatory diagnostic testing results; after application of exclusion criteria, 21 articles underwent evaluation. Aneuploidies including 45,X, 47, XXY, 47,XXX and 47,XYY were addressed in the studies analyzed. The analysis revealed a sensitivity of 98.8% (95%CI 94.6% – 100%), specificity of 99.4% (95%CI 98.7% – 99.9%) and PPV of 14.5% (95%CI 7.0% – 43.8%) for 45, X. In the cases of 47,XXY, the sensitivity was 100% (95%CI 99.6% – 100%), specificity 100% (95%CI 99.9% – 100%) and PPV 97.7% (95%CI 78.6% – 100%). For 47,XXX, the sensitivity was 100% (95%CI 96.9% – 100%), specificity was 99.9% (95%CI 99.7% – 100%) and PPV was 61.6% (95%CI 37.6% – 95.4%). Lastly, for 47,XYY, sensitivity was 100% (95%CI 91.3% – 100%), specificity was 100% (95%CI 100% – 100%) and PPV 100% (95%CI 76.5% – 100%). Estimated NPV exceeded 99% for all four of the SCAs noted, although there were some false negatives reported. Based on these results, the researchers concluded that cfDNA is a dependable test when used for screening for SCA. However, both false positive results and false negative results were reported. In addition, all pregnancies included were high-risk for aneuploidy, which limits the ability to generalize these results to the population including average risk pregnancies. Further studies in average-risk pregnancies are needed to confirm the findings of this study. Studies by Petersen et al. (2017), Pergament et al. (2014), Porreco et al. (2014), Bianchi et al. (2012), Nicolaides et al. (2013), and Sehnert et al. (2011), previously discussed in this policy, were included in this systematic review.

In a 2022 ACMG systematic evidence review, Rose et al. evaluated the performance of non-invasive prenatal screening (NIPS) in a general-risk pregnancy population. Studies which evaluated the use of NIPS for identifying Down syndrome, trisomy 18, trisomy 13 were included in the analysis along with sex chromosome aneuploidies (SCAs), rare autosomal trisomies, CNVs and maternal conditions. Studies delving into the psychological impact of NIPS and rates of subsequent diagnostic testing were also considered. In all, 87 studies met inclusion criteria and were analyzed in this assessment. For both singleton and twin pregnancies, the diagnostic odds ratios were significant ($p < .0001$) for trisomy 21, trisomy 18, and trisomy 13. The authors state: “few studies have comprehensively evaluated the use of NIPS for twin gestations. The results from our meta-analyses show NIPS performance in this population are generally comparable to performance in singleton pregnancies for T21, T18, and T13.” NIPS was also found to be accurate for detection of SCAs (≥ 99.78%). In cases of rare autosomal trisomies and CNVs, the performance of NIPs was varied. Overall, use of NIPS appears to have led to a reduction in diagnostic testing over time in the range of 31%-79%, based on various studies. Insufficient data was available to draw conclusions about psychosocial outcomes related to NIPS and the identification of maternal conditions was rare. Publications by Gil et al. (2019), Hu et al. (2019), Norwitz et al. (2019), van der Meij et al. (2019), Wan et al. (2018), Martin et al. (2018), Pertile et al. (2017), Petersen et al. (2017), Schwartz et al. (2018), Scott et al. (2018), and Bianchi et al. (2015), previously discussed in this policy, were included in the ACMG systematic review.

In a recent publication, Dar et al. (2022a) reported findings from a multicenter prospective observational study (SMART study) that compared the performance of cfDNA) screening for trisomies 13, 18, and 21 between low-risk and high-risk women. Test performance as well as no-call rates were measured, and outcomes were confirmed by follow-up genetic

testing (chromosome microarray testing [CMA] through DNA from fetal or infant samples). Confirmatory samples were obtained postnatally in all cases, regardless of whether previous prenatal diagnostic genetic testing was done. The study also assessed an updated cfDNA algorithm which was blinded to the pregnancy outcome. A total of 20,194 women across 21 centers in 6 countries were enrolled. The median gestation age was 12.6 weeks and genetic outcomes were confirmed in 17,851 cases (88.4%). Of those, 73.1% were low risk and 26.9% were high risk for aneuploidy. In total, 133 trisomies were diagnosed. As expected, positive rate was lower in the low-risk group than the high risk group (0.27% vs 2.2%) and the sensitivity and specificity were very similar between the two groups. Positive predictive value (PPV) for the low risk group was 85.7% vs 97.5% for the high risk group. No-call result after first draw was 3.4% (602) and after including cases with a second draw was 1.61% (287). A higher trisomy rate was found in these 287 individuals with a no-call result than in individuals with a result on the first draw. Updated algorithm was assessed to have similar sensitivity and specificity to the study algorithm, but the no-call rate was lower. The authors concluded that in women who are at low risk for aneuploidy, single-nucleotide polymorphism-based cfDNA has high sensitivity and specificity, a PPV of 85.7% for trisomy 21 (compared with 97.5% in the high-risk group) and PPV of 74.3% (compared with 94.2% in the high risk group) for the 3 common trisomies. Testing performed similarly for high and low risk groups. The data also indicated that individuals with no-call results are at a higher risk of aneuploidy and as such, require added investigation. The study was funded by Natera, maker of the Panorama test used in the study.

An ECRI Genetic Test Assessment (2022) evaluated the use of NIPS for identifying fetal aneuploidy risk in chromosomes 13, 18, 21 and sex chromosomes in individuals with a twin pregnancy. Overall, “somewhat favorable” evidence was found. The data used for this assessment indicated that NIPS accurately identified pregnancies in which there was a high risk of trisomy 21, potentially reducing the need for invasive testing for some individuals and had high rates of accuracy for negative test results for trisomies 13 and 18. No studies were identified which evaluated whether invasive testing rates were reduced in twin pregnancies. Unfortunately, the effectiveness for detection of trisomies 13, 18, and SCAs could not be determined due to low rate of occurrence and small study sizes. ECRI recommends continued study with large diagnostic cohort groups to determine the effectiveness of NIPS for identification of trisomy 13, 18, and SCAs in twin pregnancies as well as the clinical utility for reduction of invasive testing rates.

In a 2021 systematic review and meta-analysis, Judah et al. (included in the 2022 ECRI assessment discussed above) scrutinized the performance of cfDNA testing when used to screen for trisomies 13, 18, and 21 in twin gestation pregnancies. Data from the Fetal Medicine Foundation (FMF) as well as evidence identified in a systematic review of the literature specific to prospective first trimester screening in twin pregnancy were evaluated via meta-analysis. A total of 1,272 twin pregnancy cases from the FMF data were included in the study; of those, 20 cases were found to have trisomy 21, ten had trisomy 18 and two had trisomy 13 identified by pre- or postnatal karyotyping or by birth of a child with a normal phenotype. The cfDNA test used (Harmony prenatal test) correctly identified 95% of the trisomy 21 cases, 90% of the trisomy 18 cases and 50% of the trisomy 13 cases. The test accurately identified 99.6% of the cases that had none of the three trisomies. The systematic review resulted in identification of 12 additional pertinent studies for inclusion. In these,137 twin pregnancies with results indicating trisomy-21 and 7,507 cases without trisomy-21 were identified. Pooled, weighted DR for the trisomy-21 group was 99.0% (95% CI, 92.0-99.9%) and false positive rate (FPR) was 0.02% (95% CI, 0.001-0.43%). The pooled weighted DR and FPR were 92.8% (95% CI, 77.6-98.0%) and 0.01% (95% CI, 0.00-0.44%), respectively, in the 50 combined total cases where results indicated trisomy-18 and 6,840 pregnancies without trisomy-18. In the 11 cases where trisomy 13 was identified and 6290 cases where trisomy-13 was not found, the pooled weighted DR and FPR were 94.7% (95% CI, 9.14-99.97%) and 0.10% (95% CI, 0.03-0.39%), respectively. Based on this data, the authors concluded that the reported DR of trisomy 21 when cfDNA testing is used is high, but lower than in singleton pregnancy. The FPR, however, appears to be comparable to that found in singleton pregnancy testing. Cases of trisomy-18 and trisomy-13 cases were too limited for accurate assessment of cfDNA test performance. Publications by Norwitz et al. (2019) and Chibuk et al. (2020) which were previously discussed in this policy, and Khalil et al. (2021), discussed below, were included in this systematic review.

Khalil et al. (2021, included in the Rose et al. ACMG systematic review and the Judah systematic review) published the results of a prospective multicenter blinded study and systematic review assessing screening performance of cfDNA for detection of fetal trisomies in twin pregnancies. Primary outcome was performance/failure of cfDNA screening test using next generation sequencing (the IONA test). A total of 961 participants at least 16 years of age (276 with monochorionic twins and 685 with dichorionic twins) were included in the evaluation. The study was located in 6 fetal medicine centers in England, UK. Maternal blood was obtained during or after conventional screening tests and prospective detailed outcomes were recorded for all newborns. Failure rate for the test was 0.31%. Mean FF was 12.2% (range of 3%-36%) and all nine samples with only 3% FF provided a valid result. No false positives were obtained for either trisomy 21 or trisomy 12, but there was one false negative and one false-positive for trisomy 18. DR was 100% for trisomy 21, 0% for trisomy 18 and 100% for trisomy 13. Corresponding rates for false positives were 0%, 0.10% and 0%, respectively. The authors concluded that cfDNA screening is the most accurate screening test for trisomy 21 in twin pregnancies and has performance similar to that of singleton pregnancies with low failure rates (0.31%). Accuracy for trisomy 18 and 13 appear

to be lower, however because the FPR was low, the authors assert that first line screening with cfDNA in twin pregnancy is appropriate and should be considered a primary screening test for trisomy 21. Of note, cfDNA is unable to predict which twin is impacted by the trisomy, so nuchal translucency and detailed ultrasound would potentially be utilized to determine which twin may be impacted, thereby facilitating the choice of which fetus to test genetically. Although this study is one of the largest twin studies investigating cfDNA analysis of trisomies, a limiting factor is that there was a relatively small number of pregnancies that were affected. The study was funded by Yourgene Health Clinical Service Laboratory, maker of the IONA® test and several of the study authors are or have been employed by Yourgene.

A recent Hayes report (2021a, updated 2023) evaluated the clinical utility of cfDNA screening for trisomies 13, 18, and 21 in low-risk women with singleton pregnancies. The evaluation found limited evidence suggesting that use of cfDNA fetal screening in low-risk women as a first-tier test is likely to reduce the rate of more invasive diagnostic procedures when compared with conventional screening methods. Potential clinical benefits from increased use of cfDNA screening include a lower FPR (based on clinical validity) and a lower rate of confirmatory invasive testing, however evidence is still limited in quality and quantity for low-risk women with singleton pregnancies, specifically related to reduction in invasive diagnostic procedures.

In a Clinical Utility Evaluation, Hayes evaluated the use of cfDNA testing for fetal SCA in individuals with twin or singleton pregnancies. Per the Hayes report, published evidence suggests that using cfDNA screening for SCAs in singleton pregnancies leads to diagnostic testing for confirmation, subsequent detection of pregnancies with SCAs, and finally, use of these results to guide decision-making in pregnancy management for some women. Confirmatory diagnostic testing rates in individuals with abnormal screening results have varied, however, and it is unclear whether prenatal detection of SCAs leads to improved outcomes. Cited meta-analyses by Soukkhaphone et al. (2021) and Gil et al. (2017) have indicated that additional high quality studies are needed to evaluate the clinical performance of cfDNA for SCA in singleton pregnancies. There were no meta-analyses identified for performance of fetal SCA in twin pregnancy and only one small study addressing SCA, which showed no change in pregnancy management decision-making (Bai et al., 2022). Per this evaluation, it is unclear whether prenatal identification of SCA leads to improved outcomes with singleton pregnancy and there is insufficient evidence to form any conclusion regarding utility of CF DNA testing for fetal SCA in twin pregnancies (Hayes 2021b, updated 2023).

Another Hayes Clinical Utility Evaluation (2021c, updated 2023), assessed the use of cfDNA screening for fetal trisomy 13, 18, and 21 in twin pregnancies. The evaluation found limited data reporting the epidemiology of these three trisomies in twin pregnancies and noted that most individuals with negative cfDNA results and twin pregnancies are not likely to experience unidentified cases of trisomy 13, 18, or 21. Although there is some limited evidence suggesting that cfDNA fetal screening as a first-tier test for individuals with a twin pregnancy may reduce unnecessary invasive diagnostic procedures, overall the evidence base is limited in quantity and quality.

Hong et al. (2020) reported the results of several NIPT tests from a single center and confirmed their accuracy and reliability. NIPT data was retrospectively collected from 1,591 women from CHA Gangnam Medical Center from March 2014-November 2018. Karyotype was confirmed based on chorionic villus sampling, amniocentesis or postnatal cord/peripheral blood. Of the 1,591 women, 1,544 (97.0%) of cases were reported to have negative NIPT results and 40 (2.5%) had positive NIPT results. Overall, for chromosome abnormalities, the sensitivity and specificity were reported as 96.29% and 99.93%, respectively. The PPV and negative predictive value (NPV) were 72.22% and 99.93%, respectively. There was a decrease in the mean number of amniocentesis performed from 31.5 per month to 21.7 per month after utilization of NIPT as a screening tool. The authors concluded from their study that NIPT is an accurate and specific screening test for trisomy 21 due to its high PPV and can reduce potential procedural-related risks. Additional studies for confirmatory testing to predict cases of trisomy 13, 18, and SCA were suggested.

The Province of Ontario, Canada commissioned a health technology assessment (HTA) of NIPT in 2019 (Ontario, 2019). The HTA committee reviewed available evidence in the peer-reviewed literature, including eight studies on NIPT accuracy or clinical utility and seven studies on health care professional's understanding of NIPT, and concluded that "NIPT is an effective and safe prenatal screening method for trisomies 21, 18, and 13 in the average-risk or general population." They noted that NIPT was more accurate with an improved sensitivity and specificity than traditional forms of aneuploidy screening. Studies by Bianchi et al. (2014), Norton et al. (2015), and Nicolaides et al. (2013), previously discussed in this policy, were included in the Ontario HTA.

Dyr et al. (2019) conducted a retrospective study reporting on Sequenom's clinical laboratory experience of more than 30,000 multifetal samples (twins, triplets and higher order) using cfDNA. This was the largest to date cfDNA multigestational pregnancy study and the only known study with experience in triplets and higher-order gestation. Sequencing data was analyzed from 2011-2017 using the MaterniT21 platform to identify autosomal trisomies and other subchromosomal events. All samples were tested for trisomy 21, 18, and 13. Select, opt-in samples (58%, n = 16,951)

were evaluated for trisomies 16 and 22 and seven common microdeletions including 22q-, 5p-,15q-, 1p36-, 11q-, 8q- and 4p-. FF was adjusted in proportion to fetal number. Feedback was provided in 50 cases; six positive results for microdeletions and seven positive results for trisomies 16 were included; 6.7 (86%) reported missed abortion or co-twin demise. 4/6 positive microdeletions had diagnostic testing and there were three true positives; two who did not have diagnostic testing had suggestive clinical findings. Non-reportable rate was 5.95% and the positivity rate for trisomy 21, 18, and 13 in multifetal samples was 1.50%, 0.47%, and 0.21%, respectively. It was concluded by the authors that cfDNA for multifetal gestation offers accurate screening for fetal aneuploidy that meets and exceeds performance of original clinical validation studies. However, in this retrospective study, the absence of outcome data is a significant limitation.

The performance of cfDNA screening (QNatal Advanced) for trisomies 13, 18, 21, and SCAs was conducted on a population of both average and high risk pregnancies using massive parallel sequencing and a GC content correction algorithm. Pregnancy outcome was obtained by genetic counselors and /or Quest diagnostics client services. The mean age at delivery was 35.2 +/- 5.8 years; 69% were advanced maternal age. Twins and higher order multiples represented 2.0% and 0.02% of specimens, respectively. 69,794 specimens were subjected to cfDNA screening. 87% were high risk pregnancies; 13% were classified as average risk. 1,359 (1.9%) had a positive result. Of the confirmed cases, PPV for trisomies 21,18, 13, SCA and microdeletions was 98.1%, 88.2%, 59.3%, 69.0% and 75.0%. The overall PPV was 87.2%, sensitivity was 97.9%, and specificity was 99.9%. The authors attribute the higher PPVs obtained in this study to possible technological differences between Qnatal advanced and alternate laboratory assays. The authors also add that the high PPV for confirmed SCA outcomes (69.0%) with an incidence of 0.36% for both average and high risk patients may have been due to prospective analysis of possible maternal SCA that was performed to avoid false-positive results (Guy et al., 2019).

A 2018 Hayes report (updated 2022) evaluated clinical utility for the use of cfDNA to screen for trisomies 13, 18, and 21 in women with high-risk pregnancies and singleton or multiple gestation pregnancies. A moderate-quality body of evidence examining clinical utility supports some proven benefit, with published evidence showing that safety and health outcomes are at least comparable to standard treatment for women with singleton pregnancies. The published evidence for use of this screening in high-risk women with multiple gestation pregnancies, however, was insufficient to assess either safety or health outcomes.

Jones et al. (2018) examined the performance of cfDNA analysis of the X and Y chromosomes using microarray quantitation for determination of SCA probability in singleton pregnancy and fetal sex in twin and singleton pregnancies. Banked maternal plasma from 791 singleton and 51 twin pregnancies was collected as part of an ongoing multicenter clinical study (NCT02201862 and NCT01451671) from King's College London, UK; karyotypes were obtained for each sample. Y-chromosome specific DANSR assay was used to determine fetal sex in singleton and twin pregnancies. Results were reported based on the presence or absence of the Y chromosome fragments. For SCA evaluation, 742 samples were qualified. Fifteen cases of SCA were correctly classified (100% sensitivity; 95% CI, 79.6-100%). For the 727 disomic pregnancies, 725 were correctly identified as low risk for SCA (99.7% specificity; 95%CI, 99.0-99.9%). 748/752 singleton and 39/39 twin pregnancies had results obtained for fetal sex determination. Fetal sex was concordant with karyotypic sex in 786/787 cases (99.9% concordance). All twin cfDNA results accurately reflected either the presence of two females (n = 18) or at least one male fetus (n = 21). The authors concluded that targeted cfDNA testing was performed with high accuracy for fetal sex assessment in both singletons and twins, and correctly identified all SCAs with high specificity. However, the observed positive predictive value in this group may not be applicable to routine prenatal screening populations.

SCAs have a prevalence of about 1 in 460 in pregnancies undergoing amniocentesis, making them as common as autosomal aneuploidies in the average risk population. NIPT has limitations in identifying SCA because of placental mosaicism, fetal mosaicism, and maternal X chromosome variations. Ramdaney et al. (2018, included in the Hayes 2021b Clinical Utility Evaluation, above) retrospectively examined a cohort of 136 women with singleton pregnancies who underwent NIPT and received positive results for a SCA. The testing occurred between January 2013 to September 2017. All women had post-test genetic counseling in which the PPV of the test, its limitations, and clinical history of the associated SCA was reviewed. All patients were offered invasive prenatal diagnosis, and the benefits, risks and limitations were reviewed, and all patients had an ultrasound at the time of genetic counseling or when diagnostic testing was performed. The median patient age was 32. Seventy-three (54%) patients had NIPT positive for 45,X. Two pregnancies were fetal demises at the time of consultation, and confirmational testing was not pursued on the products of conception. Twenty-five (35%) had ultrasound findings consistent with 45,X. Two patients were lost to follow up, four decided to have prenatal diagnosis, and one of these had normal karyotype results. The remaining 19 patients with ultrasound findings declined testing. One terminated her pregnancy, eight later had a fetal demise. Ten patients are thought to have had live births, but six were lost to follow up. Four had post-natal confirmational testing that confirmed X abnormalities, including one baby with a 7:X translocation. Amongst the 46 pregnancies positive for 45,X and no ultrasound abnormalities, 24 chose prenatal diagnosis. Twenty-one had normal results, and the three abnormalities were mosaic. The other 62 patients

had positive NIPT results for 47, XXY (n = 27), 47,XXX (n = 22), 47,XYY (n = 13), and one other (48,XXYY). Fifteen women chose prenatal diagnosis, and 11 had confirmed abnormal karyotypes. In the cohort that declined invasive testing, four had ultrasound abnormalities, including one fetus with clubfoot and ambiguous genitalia. Post-natal testing identified this baby had 48, XXYY. Post-natal testing was recorded in another 13 patients. Nine of the 14 post-natal tests were consistent with NIPT results. Overall, the majority of patients declined prenatal diagnosis (65%) even when ultrasound abnormalities were present. In the 64 women with outcome information available, the NIPT result was confirmed in 30 (47%). The authors concluded that comprehensive pre- and post-test counseling is recommended, and that practice guidelines need to address provider responsibilities for post-natal testing when a positive NIPT for SCA is identified.

Mackie et al. (2017, included in the 2020 Ontario HTA discussed below in evidence) conducted a meta-analysis of cohort studies reporting on cffDNA screening results in singleton pregnancies. They reviewed peer reviewed publications identified through Medline, Embase, CINAHL, and the Cochrane Library, with publication dates from 1997 to April 2015. Inclusion criteria were singleton pregnancies of any gestation with results confirmed by karyotype or phenotype, cohort studies, and had more than five participants. Case studies, pre-implantation testing, or other fetal cell testing studies were excluded. A total of 117 studies met criteria for 18 conditions. The sensitivities and specificities for each condition were determined by bi-variate meta-analysis. For trisomy 21, there were 148,344 tests identified with a respective sensitivity and specificity of 99% and 99%. For trisomy 18, there were 146,940 tests identified with a respective sensitivity and specificity of 97% and 99%. Monosomy X had 6,712 tests identified with a respective sensitivity and specificity of 93% and 99%. RhD had 10,290 tests identified with a respective sensitivity and specificity of 99% and 98%. Trisomy 13 was evaluated by univariate analysis, and there were 134,691 tests identified with a respective sensitivity and specificity of 90% and 100%. The authors concluded that NIPT could be considered diagnostic for determining fetal sex and RhD status. However, they determined that NIPT for chromosome 21, 13, and 18 aneuploidies is a screening test because of the lower disease prevalence, role of confined placental mosaicism, and lower sensitivity and specificity.

Other Aneuploidies

The use of cfDNA for determination of aneuploidies other than trisomies 13, 18, and 21 is still in the early stages of development. Evidence at this time is limited and has shown a high number of false positive results.

To evaluate the application of NIPT for screening rare autosomal abnormalities, a study was conducted on 81,518 pregnant individuals who had undergone NIPT at a Chinese hospital between May 2018 and March 2022 (Zhang et al., 2023). Samples deemed high risk were evaluated with amniotic fluid karyotyping and CMA and pregnancy outcomes were recorded. NIPT identified 292 cases (0.36%) of rare autosomal abnormalities in the cohort. One hundred-forty of these (0.17%) were rare autosomal trisomies (RATs). Of the 140 pregnancies with RATs detected, 102 individuals agreed to invasive testing for confirmation. Five of these individuals were found to have a true positive (PPV = 4.90%). CNVs were identified in 152 samples (0.19%); 95 individuals agreed to CMA testing which confirmed true positive in 29 pregnancies (PPV = 30.53%). Clinical information was acquired in 81 of the 97 cases with false-positive RATs results. Of these, 37 (45.68%) had adverse perinatal outcomes and a higher incidence of small for gestational age babies, intrauterine growth retardation and preterm birth. Based on these results, the authors indicate that NIPT is not recommend for screening for RATs.

Hayes (2021d, updated 2023) published a Clinical Utility Evaluation addressing the use of cfDNA screening for fetal RATs in singleton and twin pregnancies. The report asserts that the use of this screening in singleton pregnancies leads to confirmatory testing is some women, but few of the women with confirmed rare autosomal aneuploidies (RAAs) used the final diagnostic results for pregnancy management decisions. Of those who underwent confirmatory diagnostics based on the RAA cfDNA screening, more than 50% of the cases were found to be false positives. Overall, the published evidence regarding use of RAA fetal testing in singleton pregnancy is very low in quality and insufficient to come to any conclusions regarding clinical utility at this time. With regard to fetal RAA testing in twin pregnancies, there were no identified peer-review studies that assessed clinical utility in individuals with twin pregnancies; evidence is thus insufficient to draw conclusions related to the clinical utility of this RAA testing in twin pregnancies.

Xue et al. (2019) conducted a retrospective evaluation of prospectively gathered NIPT information for the detection of rare fetal chromosome aneuploidy (RCA). Analysis was performed for all 24 chromosome aneuploidies among 57,204 pregnancies in Suzhou China. A total of 586 positive cases were identified following NIPT; 92 were positive for RCA. Forty-three cases underwent prenatal diagnosis and only 1 case of trisomy 22 was confirmed. Chromosome 7 aneuploidy accounted for 25/92 positive RCA NIPT results which is consistent with previous reports of trisomy 7 being the most frequently detected chromosome abnormality. Follow-up revealed that several false-positive RCA cases were caused by confined placental mosaicism, maternal aneuploidy and maternal cancer. The authors conclude that NIPT accuracy for RCAs remains limited.

Van Opstal et al. (2018) reported on the presence of rare trisomies, and other abnormalities found by the Trial by Dutch laboratories for Evaluation of Noninvasive Prenatal Testing (TRIDENT) study. The TRIDENT study was a trial where NIPT was offered as an alternative to pregnant women considering invasive prenatal diagnosis between April 2014 and April 2015. NIPT testing was performed using whole-genome shallow massively parallel shotgun sequencing. Of 3306 enrolled cases, 753 were analyzed only for chromosomes 21, 13 and 18. All others, 2553, were analyzed for all chromosomes and for segmental subchromosomal abnormalities. Results were reported in 2527 cases. In 78, a common trisomy was found, and follow up information was reported elsewhere. Forty-one cases of another type of chromosome abnormality were identified. One case of reported trisomy 8 was terminated at a private clinic before any follow up was available. In the remaining 40 cases, ten were confirmed to be true positives. These included two cases of trisomy 9, which were confirmed to be mosaic in the fetus. Both resulted in live births with multiple congenital anomalies. One case of a dual trisomy 15 and trisomy 22 was reported, and fetal tissue confirmed a mosaic trisomy 15. The pregnancy resulted in a live birth with no identifiable anomalies. One trisomy 22 was identified, confirmed as a mosaic trisomy 22. The pregnancy had multiple anomalies and was terminated. Six of the 10 cases were genomic imbalances that included dup 2p, del 6q, del80/dup8q, del 9p, del 12q, and del 18p. All were confirmed through amniocentesis. In 22 of the 40 positive cases, placental testing confirmed that confined placental mosaicism was the likely cause of the NIPT results, and in this group, there were ten infants with some impact, ranging from small for gestational age to multiple congenital anomalies. The authors conclude that genome wide screening for NIPT results in identification of chromosomal aberrations other than trisomy 13, 18, or 21 in about one-third of screen positive results, and this information is important for pregnancy management.

GENOMA Laboratory in Rome reported on their experience of offering genome wide NIPT in a general population of pregnant women in Fiorentino, et al. (2017). Their methodology relies on massively parallel sequencing (MPS) of cfDNA, followed by bioinformatic analysis for the common trisomies, and then a subsequent bioinformatic analysis for rare trisomies and segmental genomic imbalances. Samples were excluded if the FF was < 2%, or the assay failed. From December 2015 through May 2016, testing was offered to an unselected consecutive series of pregnant women seeking NIPT for common trisomies. Only singleton pregnancies qualified, and the gestational age was at least 10 weeks. Indication for testing included advanced maternal age, positive maternal serum screening, prior pregnancy with a trisomy, patients < age 35 who wanted screening, or fetal anomaly found on ultrasound. All patients were followed to determine outcome. Those with positive NIPT results had confirmational testing by CGH or karyotype. Normal results were confirmed by newborn physical or genetic testing. Overall, there were 12,114 reportable results. One hundred and sixty-six were positive for a common trisomy, including SCAs, and 151 were confirmed as true positives. The cases classified as false positive included one trisomy 21, one trisomy 18, one trisomy 13, ten monosomy X, one XXX, and one XXY. The authors concluded that the sensitivity was 100% and the specificity was 99% for the common aneuploidies. For rare trisomies and genomic imbalances, there were 30 pregnancies with a positive result. Rare trisomies were reported for 17 samples and confirmed in ten, three of which were low level mosaicisms and seven resulted in spontaneous miscarriage. One pregnancy was positive for trisomy 15, and invasive testing revealed a diploid 15 with uniparental disomy of chromosome 15 because of a rare trisomy rescue. Thirteen pregnancies had subchromosomal imbalances that was confirmed in eight cases. In two cases, the fetus was found to have an unbalanced chromosome translocation, inherited from a parent that was unaware they had a balanced translocation. The authors concluded that genome wide screening detected 12 viable pregnancies with clinically relevant abnormalities that would have been missed using standard NIPT screening.

Copy Number Variations (CNVs) and Microdeletions

The evidence is insufficient to support the use of cfDNA for the screening of microdeletions and CNVs. Further validation studies are needed to determine the sensitivity and specificity of this screening test for microdeletion syndromes.

Maya et al. (2023) sought to evaluate the theoretical value-add of two types of cfDNA screening expansions in pregnancies with no evidence of major structural abnormalities over standard cfDNA testing (13, 18, 21, X and Y) and also assess them in terms of the added value of CMA in a retrospective cohort study. The study was based on the CMA results of pregnant individuals with normal ultrasounds who had undergone amniocentesis between January 2013 and February 2022. Of the 8605 pregnancies assessed, 1.4% (n = 122) had clinically significant CMA results. Standard cfDNA testing would have theoretically identified 36.1% of these. In addition to aneuploidies detectable with standard cfDNA testing, three cases detectable with expanded cfDNA testing (including commonly found microdeletions) and nine cases detectable with genome-wide cfDNA screening (excluding common microdeletions) were identified in the overall cohort. The researchers assert that of the clinically significant CMA findings, standard cfDNA screening would miss 63.9% and genome-wide cfDNA screening would miss 54.1%. CfDNA screening expanded to include detection of microdeletions would increase value over standard cfDNA testing by approximately 0.035% and genome-wide cfDNA screening including large CNVs would result in an increase in value of approximately 0.14%; these results are far lower than the value-add of CMA (0.91%).

Several recent studies have explored the accuracy of cfDNA testing for the identification of microdeletion/microduplication syndromes (MMS) and nonsyndromic CNVs. Yang et al. (2022) assessed 19,068 singleton pregnancies that had been screened with cfDNA testing using high-throughput sequencing. Of 170 individuals whose testing revealed abnormalities, 113 (66.5%) opted for invasive testing. PPV of CNV sequencing for all types of CNVs detected in the study was 35.4% (61.5% for pathogenic MMSs and 27.6% for nonsyndromic CNVs). Although performance for MMSs was relatively high, the low PPV for nonsyndromic CNVs led the authors to conclude that the use of expanded cfDNA testing would likely increase unnecessary invasive tests and potentially lead to inappropriate terminations of pregnancy. In a prospective study assessing performance of expanded noninvasive prenatal testing (Zou et al., 2023), the PPV of the cfDNA expanded test for CNVs was found to be 51.72%. Using a clinically available genome-wide cfDNA assay (Sequenom) to test 701 pregnant individuals, Soster et al. (2023) found that when CNVs evaluated were at least 7Mb and the test was specific to specific microdeletions, sensitivity was 93.8%, specificity was 97.3%, PPV was 63.8% and NPV was 99.7% when compared to microarray testing. However, when out-of-scope CNVs were included as false negatives, the sensitivity fell to 63.8%. The authors of this study indicated that microarray testing via amniocentesis provides the most accurate and thorough assessment of fetal CNVs, but genome-wide cfDNA testing may be an option for individuals who decline or are otherwise unable to undergo invasive diagnostic testing, though diagnostic testing would still be required to confirm screen-positive results. Further study is recommended to explore potential clinical utility and assess the impact of reducing the size threshold of CNVs.

The accuracy of expanded NIPT (NIPT-plus) in the detection of clinically significant fetal CNVs was the subject of a prospective analysis of 31,260 singleton pregnancies from June 2017 to December 2020 (Xue et al., 2022). Of the 31,260 pregnant individuals who underwent NIPT-plus testing at a single hospital in Fuzhou, China, results were obtained in 31,256. High risk of clinically significant CNVs were detected in 221 individuals (0.71%). Of these, 18 refused further evaluation. Two-hundred three underwent invasive testing for prenatal diagnosis revealing 78 true positive cases and 125 false positive cases. Overall PPV was 38.42% and false positive rate was 0.40%. Where known microdeletion/microduplication syndromes were identified (n = 27), the PPVs were as follows: DiGeorge syndrome, 75%; 22q11.22 microduplication syndrome, 80%; Prader-Willi syndrome, 50%; and cri-du-chat syndrome, 50%. The remaining significant fetal CNVs (n = 175) had a combined PPV of 46.5% for CNVs greater than 10 Mb and 28.57% for CNVs of 10 Mb or less. Overall, the results indicate that NIPT screening had relatively high performance for identification of 22q11.2 microduplication syndrome and DiGeorge syndrome in this study, but low/moderate detection for other clinically significant CNVs. Further high-quality studies with larger and more diverse populations, increased depth of sequencing and improved algorithms are needed.

A 2022 (updated 2023) Hayes Clinical Utility Evaluation addressed cfDNA screening for fetal chromosome copy number variants (CNVs) in individuals with twin or singleton pregnancies. There were no peer-reviewed studies evaluating clinical utility of cfDNA screening for fetal CNVs in individuals with twin pregnancy identified. Although some evidence suggests that use of cfDNA screening for fetal CNVs in singleton pregnancies may lead to confirmatory diagnostic testing in some women, it is unknown if additional CNV testing will impact the rate of confirmatory diagnostic testing from common aneuploidy cfDNA screening. Among individuals who underwent confirmatory diagnostic testing based on the CNV cfDNA result, there was a high rate of false positive results. The current published evidence is of low quality and currently does not support conclusions regarding clinical utility.

Results of a multicenter, prospective observational study designed to assess the performance of single nucleotide polymorphism (SNP)-based cfDNA screening for detection of 22q11.2 deletion syndrome were published by Dar et al. (2022b). The study also assessed prevalence of 22q11.2 deletion syndrome and the performance of an updated cfDNA algorithm which the researchers blinded to the pregnancy outcome. Enrollees from 21 centers in 6 countries participated, undergoing SNP based cfDNA screening specific to 22q11.2 deletion syndrome. Either prenatal or newborn DNA samples were requested in all cases, so that genetic confirmation using chromosomal microarrays could be performed. A total of 20,887 individuals were enrolled and a genetic outcome was available for 87.6% (18,289). Twelve 22q11.2 deletion syndrome cases were confirmed in this cohort (including 5 nested deletions), which yielded a prevalence of 1 in 1524. Of the total cohort, cfDNA screening identified 17,976 cases as low risk for 22q11.2 deletion syndrome and 38 cases as high risk, with 275 cases non-reportable. Ultimately, 9 of 12 cases of 22q11.2 were identified, equating to a sensitivity of 75%, a specificity of 99.84% and a positive predictive value of 23.7%. Negative predictive value was 99.98%. No cases with non-reportable outcome resulted in a diagnosis of 22q11.2 deletion syndrome. The updated algorithm identified 10 of 12 cases and led to lower false positive rate and an increased positive predictive value of 52.6%. The authors concluded that cfDNA screening for 22q11.2 deletion syndrome can detect most affected cases with a low false positive rate and has the ability to detect smaller, nested deletions. However, the overall confirmed number of cases of 22q11.2 deletion syndrome in this study was low, liming the ability to accurately calculate PPV as stratified by risk factors. In addition, estimates of DR for rare conditions are associated with wide confidence intervals and finally, there were varied indications for testing and prevalence rates may not reflect risk in the average population. In addition, the study was funded by Natera, the maker of a test including 22q11.2 microdeletion screening.

Cui et al. (2019) evaluated the clinical utility of non-invasive prenatal testing (NIPT) for the detection of copy number variants (CNVs) by reporting on 161 pregnancies with ultrasound findings and negative NIPT results for chromosomal aneuploidy. Fetal CNVs were diagnosed by CNV sequencing; fetal and parental karyotypes were obtained by G-banding. NIPT revealed 11 CNVs ≥ 1Mb in nine samples, including two CNVs in each one of two separate samples. CNV sequencing on amniotic fluid was performed for 137 samples and 24 samples of fetal tissue. Fetal karyotypes were obtained for 78 cases and seven cases were diagnosed as abnormal. The sensitivity and specificity of NIPT for detecting CNV > 1Mb were 83.33% and 99.34%, respectively. The PPV and NPV were 90.91% and 98.68%, respectively. The sensitivity and specificity for CNVs 1Mb-5Mb was higher than for those ≥ 5Mb. The authors claimed that NIPT can be performed for pregnancies with structural fetal anomalies for CNV detection, however due to the residual chromosomal aneuploidy risks for pregnancies with soft ultrasound markers, women with structural ultrasound anomalies should be offered invasive procedures for diagnosing CNVs. This study is difficult to generalize to the average screening population, as only pregnancies with ultrasound anomalies and negative NIPT results were selected for analysis. Future studies are needed for NIPT and CNV detection.

DiGeorge syndrome, also known as velocardiofacial syndrome or 22q11 deletion syndrome, is one of the most common microdeletion syndromes with an incidence of 1 in 3,000-6,000 births. Affected individuals have a wide array of clinical manifestations, including congenital heart defects, immune dysfunction, hypocalcemia, mild-to-severe learning disabilities, and an increased risk of mental health disorders. Ravi et al. (2018) reported on the clinical validity of using a SNP based NIPT assay to detect fetal 22q11.2 deletions during pregnancy. Women from six prenatal centers were enrolled in the study and were undergoing invasive prenatal diagnosis for a variety of reasons. At the time of blood draw, information about gestational age, maternal age and weight, and time between the invasive procedure and blood draw were collected. Samples from patients that were < 9 weeks gestation, had a fetal demise, had atypical 22q distal deletions on invasive testing, or equivocal invasive test results were excluded. Patients with inconclusive or no call NIPT results were excluded and no redraws were requested. The study was internally blinded, but ultimately included ten patients with confirmed fetal 22q11.2 deletions and 390 with unaffected pregnancies. The mean age was 28, and the gestational age averaged 21 weeks for affected pregnancies and 12.8 weeks for unaffected pregnancies. Samples were tested at Natera using a massively multiplexed PCR (mmPCR) amplification targeting SNPs covering chromosomes 13, 18, 21, 22, X, and Y. The target set contained 13,926 distinct genetic loci, including 1,351 SNPs spanning a 2.91 Mb section of the 22q11.2 region that constitutes approximately 87% of all deletions detected in individuals with the 22q11.2 deletion syndrome. Risk status for the 22q11.2 deletion was assigned as high or low risk, or risk unchanged/no call. High-risk calls with maternally deleted haplotypes were sequenced at a higher depth of read to confirm high-risk status. For cases with an FF of 2.8-6.5%, the sample was evaluated only for the presence or absence of the paternally-inherited haplotype. Of the ten affected pregnancies, nine were identified as test positive, or high risk. Of the 390 unaffected samples, one false positive was found. Overall, the study found the sensitivity to be 90%, the specificity to be 99.7%, and based on a prevalence of 22q11.2 deletions of 1 in 1,442 in pregnancy, the estimated positive predictive value (PPV) is 19.6%.

Lo et al. (2016) developed a segmentational algorithm in their NIPT bioinformatics calling pipeline to identify subchromosomal abnormalities. Maternal blood samples were collected from women undergoing invasive procedures for clinical indications in 40 maternity clinics around the UK as part of the RAPID (Rapid Accurate Prenatal Noninvasive Diagnosis) project, and a subset of samples with known outcomes were selected for use in a proof of concept study. This included 31 test samples with known unbalanced chromosome rearrangements and 534 samples with known normal chromosomes by karyotype or fluorescent in situ hybridization (FISH). After adjusting the algorithms and using a deeper read depth, 29 of 31 subchromosomal abnormalities were correctly identified. In the 534 normal samples, there were two false positive results. The authors noted that when using the standard read depth utilized in NIPT testing, only rearrangements > 6Mb could be found, and few < 6Mb unless maternally inherited. They concluded that because standard NIPT can only detect the larger chromosomal rearrangements and requires knowledge of FF, it is not yet ready for routine clinical implementation.

Gross et al. (2016) evaluated the ability of a SNP based NIPT test to detect 22q11 deletion syndrome in a commercial lab. A retrospective analysis was performed for 21,948 consecutive samples for fetal aneuploidy and microdeletion screening received over a 6-month period from February to August 2014. Demographic information received included indication for testing, gestational age, maternal date of birth, maternal weight, and whether the mother was a known microdeletion carrier. A paternal sample was requested but not required, and 5,912 (26.9%) cases included a paternal sample. Prior to analysis of 22q11.2, the standard panel testing for aneuploidy at chromosomes 13, 18, 21, X, and Y was conducted; samples that failed quality control at this step were not evaluated for 22q11.2. This region was analyzed by 672 SNPs targeting the 2.91-megabase (Mb) loci associated with the 22q11.2 deletion syndrome. Fetal results were predicted based on the pattern of SNPs, FF and paternal results when available. Ninety-five cases were reported as high risk. Of these, 84 had some outcome data available. Invasive testing was performed in 48 cases, and 11 had post-natal testing, and testing was declined by the remaining patients. Of those with follow up diagnostic testing, 11 were true positives, and 50 were false positives. Seventy-seven high risk patients had ultrasound data available, and 26 had anomalies observable on

ultrasound, of which nine were true positives. There were three pregnancy terminations related to screening results of 22q11.2 deletion, two of which were confirmed as true positive. The authors conclude that the availability of genetic counseling and other resources to manage high risk 22q11.2 cases is an important aspect of this screening test.

While individually rare, subchromosomal abnormalities occur in 1.6% of pregnancies. Helgeson et al. (2015) reported on the development of an algorithm to be applied to cffDNA testing to support identifying 5pdel, 22q11del, 15qdel, 1p36del, 4pdel, 11qdel, and 8qdel in routine testing. Low coverage whole genome massively parallel sequencing was used to analyze cffDNA and used a statistical method to search for consistently under-represented regions followed by a decision tree to differentiate whole-chromosome events from regional deletions. A cohort of 175,393 high risk pregnancies was used to test the algorithm. Samples were collected from October 2013 to October 2014. Fifty-five cases were screen positive for subchromosomal events. Outcome data was available for 53 cases. Chromosome microarray or FISH confirmed the findings in 41 (77%) cases. Nine cases did not have confirmational testing but had clinical features on ultrasound consistent with the deletion. Three cases were false positives. The false negative rate and sensitivity were not conclusively determined.

Fetal Antigen Testing

There is a lack of evidence in the peer-reviewed literature supporting use of cfDNA testing to determine fetal antigen status in pregnant individuals who are alloimmunized for specific antigens. Additional study, including high quality trials focused on impact to clinical outcomes, is needed.

Alford et al. (2023) used quantitative counting template (QCT) technology in the development of an NGS-based fetal cfDNA screening test that was then used to identify RhD, C, c, K (Kell), and Fy^a (Duffy) fetal antigen genotypes present in the blood of pregnant individuals in an ethnically diverse population within the U.S. The use of QCT was leveraged to allow for highly specific and sensitive quantification and identification of paternally derived fetal antigen alleles in cfDNA. In an analytical validation including 1061 preclinical samples, use of the test to determine fetal antigen status was found to have sensitivity of 100% (95% CI 99-100%) and specificity of 100% (95% CI 99-100%). An independent evaluation of two duplicate plasma samples was performed for 1683 clinical samples which showed precision of 99.9%. The “no results” rate (in clinical practice) was 0% for 711 RhD-negative non-alloimmunized pregnant individuals and 0.1% for 769 cases where the individual was alloimmunized. Clinical validation demonstrated that fetal cfDNA testing results were 100% concordant with neonatal antigen genotype serology results in a corresponding 23 RdD-negative pregnancies and 93 antigen evaluations in 30 alloimmunized pregnant individuals. The researchers concluded that the NGS-based fetal antigen cfDNA testing has the potential to detect more fetuses at risk for hemolytic disease than the standard practice which relies on paternal genotyping and invasive diagnostics, therefore limiting results due to lack of adherence or faulty attribution of paternity. The authors proposed that integration of fetal cfDNA testing for the detection of fetal antigens into care for both alloimmunized and RhD-negative non-alloimmunized pregnant individuals could increase efficiency in prenatal care and potentially reduce unneeded treatment and supervision. While this data shows promise, additional high-quality studies measuring the impact of this testing on clinical outcomes is needed. In addition, the majority of authors of this study are noted to be affiliated with a fetal cfDNA test manufacturer, creating risk of bias.

In a 2023 publication, Clausen and van der Schoot focused on the use of fetal cfDNA testing for blood group antigen genotyping as a diagnostic tool for the predication of hemolytic disease of the fetus and newborn in pregnancies where the pregnant individual was immunized. The authors indicate that these noninvasive tests have demonstrated high performance accuracy and predict that the use of cfDNA fetal blood group antigen genotyping will soon be expanded in clinical practice. Anticipated challenges include the use this testing in mixed ethnic populations and the need for improvement of care in many low-income countries around the world.

Fetal Demise/Missed Abortion

The use of cfDNA from maternal blood to identify chromosome abnormalities in fetal demise or missed abortion is currently not supported by the peer-reviewed, published evidence. Additional studies are necessary to investigate variables which may impact results and provide support for clinical utility.

The feasibility of using cffDNA to differentiate euploid and aneuploid pregnancy loss was explored in a 2023 prospective cohort study (Schlaikjær Hartwig et al.) Because the likelihood of a subsequent successful pregnancy is greater with fetal aneuploidy than euploid pregnancy loss (in which an underlying maternal condition may be the cause), ploidy diagnostics have the potential to improve clinical management of individuals who have experienced pregnancy loss. Participants were part of the Copenhagen Pregnancy Loss study, which included three gynecological clinics in Denmark. Requirements for participation included age greater than 18 years and a pregnancy loss prior to gestational age of 22 weeks. Intrauterine pregnancy was confirmed by ultrasound results (no molar pregnancies or pregnancies of unknown location were included). Blood samples were obtained either while the pregnancy tissue remained in situ or within 24 of the passing of

pregnancy tissue. CffDNA was evaluated via genome-wide sequencing, with direct sequencing of pregnancy tissue performed for reference. A total of 1000 individuals participated overall; assessment of the validity of the cffDNA-based testing was based on results from the first 333. Of those 333 individuals, 105 were unable to collect pregnancy tissue or collected a sample with unknown tissue which was at high risk of being maternal. The additional 667 individuals were included with the first 333 in the larger cohort of 1000 for assessment of cffDNA performance and result distribution. The cffDNA test was determined to have a sensitivity of detection of aneuploidy of 85% (95% CI 79-90) and a specificity of 93% (95% CI 88-96) when compared with the results of direct sequencing of the pregnancy tissue. Euploid results were found in 446 cases (45%), aneuploid results were found in 405 cases (41%), and multiple aneuploidies were found in 37 cases (4%). One hundred-twelve results (11%) were inconclusive. The authors propose that these results highlight the potential for use of cffDNA for fetal ploidy evaluation after pregnancy loss and may help initiate changes in management of individuals with pregnancy loss that lead to better outcomes for affected individuals and their families. Additional high-quality studies are needed to further validate these findings and establish clinical utility.

In a prospective diagnostic test study, Yaron et al. (2020) sought to determine whether cfDNA could accurately detect chromosome abnormalities in early pregnancy loss and recurrent pregnancy loss. A total of 109 participants experiencing early pregnancy loss (prior to 14 weeks gestation) consented to participate. Of these, 97 cases had cytogenetic results available from POC testing. Cases showing mosaicism (n = 9) were not included in the analysis and in 2 situations, no cfDNA results were available. Gestational age ranged from 5.1 to 13.6 weeks and median maternal age was 37 years. Final analysis included 86 cases with eligible cytogenic and cfDNA testing results. cfDNA testing was performed using the Verifi® Plus prenatal aneuploidy screening test. The median FF in the cfDNA tests was 5%. A chromosomal abnormality was found in 64% of cases evaluated, with rate of abnormality increasing with maternal age. Using standard log-likelihood ratio thresholds, sensitivity of cfDNA for detection of aneuploidy was 55% and specificity was 100%. Additionally, the researchers used pregnancy loss-specific log-likelihood ratio thresholds established via the first 50 cases in this study; using these thresholds, the sensitivity of cfDNA for detection of aneuploidy was 82% with a specificity of 90%. Ultimately, they concluded that genome wide cfDNA testing could be used as an alternative to genetic analysis of POC in early or recurrent pregnancy loss since detection of chromosomal abnormalities could reduce or remove the need for further testing. Several limitations were noted, including the small size of the study and a significant issue presented by false-positive results of cfDNA testing; those individuals would not receive the indicated RPL workup. Although a lower FF cutoff of 4% might improve specificity, a large number of cases (> 25%) would be excluded with this cutoff.

Colley et al. (2020) explored the use of cfDNA to assess for chromosome anomalies in the case of miscarriage, asserting that identifying whether a chromosome abnormality was the underlying cause of the pregnancy loss is important in terms of prognosis of potential future pregnancies. In this study, blood samples from 102 women over 16 years of age who were going through a first trimester pregnancy loss were obtained (mean gestational age was 7.1 weeks) and Illumina VeriSeq NIPT v2 was used to perform cfDNA testing. POC were collected as well; targeted quantitative fluorescent PCR (QF-PCR) and CMA was performed on POC after third and subsequent consecutive pregnancy loss. Overall, 64 of the pregnancies had a cytogenic result from POC analysis. A total of 21 POC samples were unable to be tested and a related POC sample was not received for 17 of the cfDNA samples. The analysis of CfDNA results was performed only on the 64 samples with a usable POC sample. Known triploid pregnancies were also excluded (n = 7). In the remaining 57 cases, chromosome anomalies were found in the POC evaluation in 27 samples (47%). VeriSeq accurately identified 70% (40/57) of the samples including 16/57 with genetic abnormalities and 27 genetically normal samples, which equates to a sensitivity of 59%, specificity of 90% and accuracy of 75% in this notably small cohort. The researchers state that in some cases, cfDNA can be useful for detection of genetic abnormalities in cases of miscarriage when the sample is collected when the pregnancy tissue remains in situ and there is enough FF. However, more study is required to refine this testing and account for variables impacting overall results before this testing can be applied clinically.

Fetal Fraction (FF)

The proportion of fetal cfDNA is called the fetal fraction (FF) and is an important aspect of NIPT testing. If FF is too low, an NIPT result cannot be accurately determined. FF can be impacted by maternal weight, gestational age, and fetal aneuploidy. To be accurate, cfDNA screening requires a minimum FF, which has been most commonly estimated at 2-4% (ACOG/SMFM, 2020). Repeating an NIPT due to low FF is controversial, as before 20 weeks of gestation, the FF increases < 0.1% per week (Gregg et al., 2016). There is insufficient evidence to support the use of repeat NIPT screening due to low FF results. The limited studies available have not provided clinical utility to support this testing.

In an effort to identify maternal features that may be related to low FF results in cfDNA testing, ascertain the proportion of individuals for whom repeat cfDNA testing is successful, and detect any meaningful associations between low FF and pregnancy outcomes or interventions, Creswell et al. (2023) led a retrospective observational study at an Irish maternity hospital from January 2017 to December 2022. The study included 4465 individuals, all of whom underwent antenatal screening using targeted cfDNA testing. Participants whose first cfDNA tests did not yield a result were evaluated in two cohorts; those for whom subsequent sampling yielded a result and those for whom no result was ever achieved, despite

up to four cfDNA tests. The researchers found that maternal BMI elevation and in-vitro fertilization (IVF) were associated with a substantial increase in FF insufficiency risk (odds ratio [OR] 1.07; 95% CI 1.01-1.13, p = 0.03 and OR 3.4; 95% CI 1.19-9.4, p = 0.02, respectively). Individuals who test yielded no result were more likely to undergo invasive diagnostics (p < 0.01), but their risk of aneuploidy was not elevated. A significant association was found between repeated cfDNA testing failure and later development of hypertensive diseases of pregnancy (p = 0.03). Over 70% of individuals whose first or second attempt at cfDNA screening failed due to low FF obtained a result from a second or third test. This analysis reinforces the existing evidence showing increased maternal BMI and IVF are associated inversely with FF and contributes to the evidence supporting repeat testing when cfDNA screening is initially unsuccessful. However, the study was limited by its retrospective design and the low volume of individuals with no results because of low FF. Additional prospective study including more diverse populations is needed to further evaluate conditions associated with low FF and the value of repeat testing.

Becking et al. (2023) sought to evaluate the variability in FFs reported on individual samples between providers and laboratories and within a single laboratory. Results were compared across laboratories and then stratified by test methodologies. Each sample was sequenced repeatedly and 2 bioinformatic methods were used to estimate FF; Veriseq2 and SeqFF. Lastly, the FFs were compared in a total of 87,351 samples. The researchers found great variability in reported FF, standard deviations (SDs) and coefficient of variation (CVs) which ranged from 1.7% to 3.6% and 17.0% and 35.8%, respectively. When FF was measured by SNP-based methods, there were small SDs (0.5% to 2.4%) in comparison with WGS-based methods (1.8% to 2.9%). SDs were similar between SeqFF and Veriseq2 but the Veriseq mean FF was higher when compared to SeqFF (9.0% vs 6.4%, (p < 0.001). In the collected samples, FF averaged 1.12%-points higher in Veriseq than in SeqFF. The authors concluded that standard testing methods currently do not allow for dependable and stable FF estimates. In addition, FF results should be interpreted in the context of laboratory specific ranges and not an identified standard value. The researchers advocate against the use of strict and universal minimum thresholds which could result in test failure and encourage the development of new algorithms which model uncertainties in FF estimation and other parameters into screening assay results to obtain reliable NIPT tests with low rates of failure.

In a systematic review, Scheffer et al. (2021) addressed the association of low FF in prenatal cfDNA testing with adverse pregnancy outcomes. The authors note that low FF is an important cause of test failure in cfDNA testing and has been reported to potentially be responsible for up to 6.1% of test failure rates. Fetal aneuploidy has been associated with low FF as well. Literature search was conducted using MEDLINE and EMBASE including dates up to November 1, 2020. In total, five studies met criteria for inclusion in the review with cohort sizes from 370 to 6375 pregnancies. All pregnancies had undergone cfDNA testing in the first or early second trimester. Regarding cutoff for low FF, two studies used 4%, two studies used the 5th and 25th percentiles and one study used a variety of varying cutoff values. Associations with low FF in prenatal cfDNA testing included hypertensive disease of pregnancy, small for gestational age newborns and preterm birth. Results for association of Low FF and gestational diabetes mellitus were conflicting. The researchers concluded that low FF in cfDNA testing is associated with adverse pregnancy outcomes including pregnancy-related hypertensive disorders, preterm birth and impaired fetal growth related to placental dysfunction, in addition to its previously established association with fetal aneuploidy, and recommended that if gestational age allows, individuals with cfDNA test failure due to low FF should be advised to consider repeat testing from an additional blood draw. However, this review had limitations, including the limited number of publications available that addressed the relationship between low FF and adverse pregnancy outcome (five), and the relatively small sample sizes, which do not allow generalization to the larger pregnant population. In addition, four of the five studies included women of advanced maternal age, which could increase the rate of adverse pregnancy outcomes and three of the studies included women with pre-gestational conditions such as chronic hypertension and diabetes. The FF cutoff also differed between the various studies so results may not have been consistent between the groups. Ultimately, the authors recommend further prospective research in large cohorts with continuous values of FF to allow for determination of cutoff values associated with increased risk of adverse pregnancy outcomes.

Benn et al. (2019) reviewed 159,574 Natera SNP-based NIPT samples between January 1, 2016, and October 1, 2016, to identify cases with a “no call” result and that underwent subsequent redraw. The dataset included 2,959 cases with a no-call result due to low FF. Risks for trisomy 13, 18, and triploidy were evaluated using an FF-based algorithm. For each sample, an FF z-score (number of SDs that the FF departed from the mean after adjustment for patient weight/gestational age) and a fetal fraction-based risk (FFBR) score were calculated. Using a risk cut-off of 1/100, the FFBR algorithm was introduced into clinical practice and provided an increased risk for the relevant condition as opposed to reporting an uninformative NIPT result; genetic counseling, comprehensive ultrasound and prenatal diagnosis were then recommended. Women receiving an FFBR risk < 1/100 do not demonstrate an increased risk and can be offered the option of repeat testing. Risk-unchanged women were subsequently analyzed with a different regression model to determine the likelihood of an informative redraw. Of 2,644 samples with an uninformative result and redraw, 1,147 (43.4%) were high risk for trisomy 13,18 or triploidy. 1,497 (56.6%) were risk unchanged and of these, 975 (65.1%) had an informative redraw (80% of the original no-calls had informative results). Initial FF, maternal weight and time between

blood samples were directly related to the likelihood of a successful redraw ($p < .001$). Using the revised FFBR algorithm which uses FF as a biomarker after adjustment for maternal weight and gestational age for women with unchanged results, the number of women with an uninformative NIPT result was reduced to 0.7%. This study had several limitations including the dataset as a whole. The analysis is based on high-risk women with suspected chromosome abnormalities and the actual pregnancy outcomes are unknown for the cases in this study. The model has not been validated in a prospective study group. The authors concluded that this new algorithm and predictive model may be informative for redraw information for women with low FF and uninformative NIPT. Additional studies with outcome analysis are needed.

White et al. (2019) retrospectively studied factors that influence obtaining results on repeat NIPT following insufficient FF from initial specimen. A total of 2,906 samples were submitted for repeat testing by Harmony NIPT assay. Maternal age, weight, gestational age, time of sample, method of conception and number of fetuses were ascertained. An FF with a minimum of 4% was required. A result was obtained in 53% of subsequent specimens. The likelihood of obtaining a result was associated with the interval time between draws (per day, OR 1.040, 95% CI 1.031-1.051) and maternal weight (per kg, OR 0.988, 95% CI 0.985-0.991). An association was not made based on maternal age, gestational age at testing, IVF status or twin/singleton status. For every day the redraw interval increases, it is expected to see a 4% increase in the likelihood of obtaining a result. However, there is a 1.2% decrease in the likelihood of obtaining results for every kg increase in the maternal weight. A total of 246 pregnancies were redrawn more than once with 51.0% reporting results with the second submission. The authors acknowledged that the study findings should be applied after a normal ultrasound has been confirmed. No information was available about karyotype/fetal outcomes. Furthermore, the authors concluded that the decision to redraw should take into consideration ultrasound findings, other screening results, maternal factors, gestational age and parental preferences for follow-up.

McKanna et al. (2019) evaluated the role of low FF in identifying pregnancies at increased risk for trisomies 13 and 18, as well as triploidy. The authors used data from > 165,000 singleton pregnancies to construct an algorithm (FFBR) using maternal weight, gestational age, and FF distributions from normal and affected pregnancies. The algorithm was validated on a blinded set of SNP-based NIPT results from 1148 cases that had no-call results, as well as outcome data available. The average age of the cohort was 34, the average weight was 208 pounds, the average gestational age was 12.3 weeks, and the average FF was 3.1%. The primary reason for referral were advanced maternal age (55%), routine screening for average risk women (33%), abnormal maternal serum screen (4.2%), abnormal ultrasound (3.7%), or a positive family history (3.2%). FFBR scores were calculated for the cohort, and 49% (564/1148) had a high score associated with a $\geq$ risk of trisomies 13, 18, or triploidy. The rate of scores differed between referral groups as follows: advanced maternal age, 63.7%; maternal serum screen positive, 41.7%; abnormal ultrasound, 39.5%; family history, 40.5%; and the average risk group, 28.3%. Based on the characteristics of the high FFBR score cohort, the expected rate of trisomy 13, trisomy 18 and triploidy was expected to be 0.7%, however the observed rate in this group was significantly higher at 5.7%. In the low FFBR score cohort, the incidence was not significantly different at 0.5% than was expected (0.2%). Unexplained pregnancy loss was also higher in the high FFBR cohort at 14.7%, compared to what was expected of 10.4%. In this cohort, the positive predictive value of the high FFBR score was 5.7% with a sensitivity of 91.4% for trisomy 13, trisomy 18, and triploidy.

To determine if a redraw is useful when no result is obtained on an initial NIPT test, Benn et al. (2018) performed a retrospective review of samples referred to Natera laboratories between January 2016 and October 2016 that were considered to be 'no result.' The lab uses a SNP based approach to NIPT analysis and classifies tests as 'no result' when the FF is less than 2.8%, or the FF was less than 7% and SNP patterns could not be interpreted with a high degree of confidence. Of the 242,607 samples received in this time period, there were 8,605 cases that did not receive a result. Of these, 3,355 redraw samples were received. Cases that had no result because of inadequate sampling, because the test was cancelled, or for certain findings, such as large regions of homozygosity, were excluded from review. Cases that met the criteria for a redraw due to low FF or low confidence in the SNP pattern were analyzed and included 2,959 samples. A result was obtained in 1,861 (62.9%). Fetal fraction was generally higher at the time of redraw, which was, on average, 14 days after the first sample. When the initial FF was 1.5-2%, the informative redraw rate was 27.8%. If it was > 4%, the informative redraw rate was 86.5%. The authors also looked at maternal weight as a factor in a successful redraw and noted that the informative redraw rate for women < 180 pounds was 73%, and for women > 240 pounds was 47.7%. Regardless of maternal weight, the initial FF percent was the most informative for determining redraw success. The authors highlight the ACOG recommendation that women whose initial test results are not reported should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy but does not preclude repeat NIPT as an option. In contrast, the ACMG states that a repeat blood draw is not appropriate and diagnostic testing should be offered. The researchers conclude that their data provides the option of using gestation age, maternal weight, and initial FF to further inform women about the possibility of an informative redraw when considering repeating NIPT, doing maternal serum screening, or having an invasive test.

Placental mosaicism is the primary cause of false positive results in NIPT analysis. Brison et al. (2018) reported on their efforts to develop a method to detect placental chromosomal mosaicism using cell-free fetal DNA by combining FF with genome wide aneuploidy detection. A consecutive non-selected series of 19,735 pregnant women who were at least 10 weeks gestation, were tested for common fetal aneuploidies by NIPT. The categories of pregnant women included average risk (61.7%), advanced maternal age (over 36 years of age) (24.2%), increased risk for fetal aneuploidy as indicated by FTS (12.2%), familial history of congenital or hereditary diseases (1.8%), or NIPT following pre-implantation genetic diagnosis and in vitro fertilization (0.1%). A retrospective analysis of the cohort for placental mosaicism was conducted by collecting genome wide read counts that were normalized, GC corrected and aggregated per chromosome to produce a chromosomal read count (CRC). Calls were determined for all chromosomes for trisomy, monosomy, borderline aneuploidies, fetal sex and maternal copy number variants. Samples with undetermined fetal sex or poor quality were excluded. FF was estimated using the SeqFFA formula. Placental mosaicism was predicted using an algorithm incorporating the mean CRC, SD CRC with the FF. Respectively 3.2% (5/154), 12.8% (5/39), and 13.3% (2/15) of trisomies 21, 18, and 13 were predicted and confirmed to be mosaic. The incidence of rare autosomal trisomies was ~0.3% (58/19,735), 45 of which were predicted to be mosaic. Twin pregnancies with discordant fetal genotypes were predicted and confirmed.

Hudecova et al. (2014) investigated if differences in fetal DNA fractions existed between different pregnancy risk groups, as FF is an important factor in determining the reliability of the NIPT analysis. Seven hundred and twenty-six pregnant women were consecutively recruited from the Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, between November 2011 and January 2013. The inclusion criteria were male singleton pregnancies with recorded data for risk group stratification. The high risk (HR) group was defined as those pregnancies with a risk of trisomy 21 was greater than 1 in 250, the intermediate risk (IR) group had a with risk between 1 in 250 to 1 in 1000, and the low risk group (LR) had a risk of trisomy 21 lower than 1 in 1000. In total 337 cases met criteria. Statistical analysis found that a minimum of 127 cases were needed for analysis, so the authors selected 138 LR cases and all HR and IR for study, totaling 195 pregnancies. Fetal DNA fractions were compared between the three fetal aneuploidy risk groups (HR, IR, LR) and assessed for correlation with the FTS test parameters; gestational age, crown-rump length (CRL), free b-subunit of human chorionic gonadotropin (free bhCG), nuchal translucency, pregnancy-associated plasma protein A (PAPP-A) as well as maternal weight. The authors found no statistically significant difference in fetal DNA fractions across the high, intermediate and low risk groups. FF showed a strong negative correlation with maternal weight, and weak but significant correlations with gestational age, crown-rump length, multiple of medians of free b-subunit of human chorionic gonadotropin and pregnancy-associated plasma protein A. The researchers conclude that the aneuploidy screening by NIPT is likely to offer similar analytical reliability without respect to the a priori fetal aneuploidy risk because of similar fetal DNA fractions in maternal plasma between high, intermediate and low risk pregnancies in the general population.

RhD Genotyping

There is a lack of evidence to support the use of cfDNA testing for determination of fetal RhD genotyping. Limited studies have been performed and the study populations are lacking diversity.

In a 2021 systematic review and meta-analysis, Alshehri & Jackson evaluated the application of cfDNA for fetal RHD genotyping in conjunction with quantitative maternal alloantibody analysis for early diagnosis of pregnancies at risk of hemolytic disease of fetus and newborn (HDFN). A total of 19 studies from January 2006 to April 2020 were included in the analysis. The researchers found that cfDNA testing was highly sensitive and specific (as early as 11 weeks gestation) with regard to early RHD genotyping with a preference for high-throughput platforms and feel that this evidence supports the inclusion of cfDNA testing along with maternal alloantibody quantitation in routine pregnancy management. They note that knowledge of parental ethnicity is key for correct interpretation of cfDNA results and quantitative screening results and that cfDNA testing would lead to less anxiety and inconvenience for pregnant individuals. The authors conclude that future large-scale studies evaluating cfDNA non-RHD genotyping including varying ethnic groups and with the presence of clinically significant alloantibodies are needed.

A 2020 Ontario HTA evaluated the accuracy, clinical utility and cost-effectiveness of noninvasive fetal RhD blood group genotyping for RhD-negative pregnant individuals. The evaluation included a literature search which identified six systematic reviews addressing test accuracy and 11 studies addressing clinical utility. Test accuracy was found to be high across all the systematic reviews and indicated that implementation of fetal cfDNA testing for RhD genotype could lead to avoidance of unneeded RhIG prophylaxis (GRADE: Low), good compliance with targeted RHIG prophylaxis (GRADE: very low), and high uptake of genotyping (GRADE: low). In addition, alloimmunization may not increase with the use of fetal cfDNA RhD genotyping for targeting prenatal RHIG prophylaxis and unnecessary monitoring and invasive procedures in alloimmunized pregnant individuals may be reduced (both GRADE: very low). The HTA concluded that overall, fetal cfDNA testing for fetal RhD blood group genotyping is an accurate test to detect RhD incompatibility and help steer management of RhD-negative pregnancies, but only low to very low quality evidence was identified to indicate that fetal cfDNA testing for RhD genotype would lead to the avoidance of unnecessary RhIG prophylaxis, high compliance with

targeted RhIG programs and high uptake of genotyping. Studies by Yang et al. (2019), Saramago et al (2020), and Mackie et al. (2017), discussed in evidence, were included in this HTA.

A prospective cohort, systematic review and meta-analysis was performed by Yang et al. (2019, included in the 2020 Ontario HTA discussed above) to assess the diagnostic accuracy of high-throughput NIPT for fetal RhD status in RhD-negative women not known to be sensitized to the RhD antigen. Databases scanned for this meta-analysis included MEDLINE, EMBASE and Science Citation Index and were searched through February 2016. Included for review were 3,921 identified studies. The study population included RhD negative pregnant women known to not be sensitized to the RhD antigen and the index test was high-throughput cfDNA on maternal plasma. Serological cord blood testing at birth was considered the reference standard and eligible studies were required to report diagnostic accuracy data including true positive, false positive, true negative and false negative absolute numbers. Diagnostic accuracy of NIPT varied by gestational age with data suggesting that NIPT was consistently accurate any time after the first trimester. The false negative rate (those incorrectly classified as RhD negative) was 0.34% (95% CI 0.15-0.76) and the false positive rate (incorrectly classified as RhD positive) was 3.86% (95% CI 2.54-5.82). Because this study is a meta-analysis, the authors described the risk of bias in the original articles and several of the included studies were deemed to be high-risk for bias due to the selected populations and the reference standards. The authors concluded that the use of NIPT for fetal RhD screening in all RhD-negative women is possible. Results would significantly reduce the need for unnecessary prenatal anti-D prophylaxis, while marginally increasing the risk of sensitization due to false negative results.

Manfroi et al. (2018) performed fetal RhD genotyping with polymerase chain reaction using cfDNA from maternal plasma to determine the diagnostic accuracy of non-invasive fetal genotyping at different gestational ages. A commercial multiple-exon assay was used to determine the accuracy of fetal RhD genotyping. Samples from RhD negative women (n = 367) with RhD positive partners or partners with unknown RhD phenotype were collected between 24-28 weeks gestation; due to lack of available first trimester samples the analysis was restricted to 24-28 weeks during which fetal genotyping is usually performed for prenatal RhIG administration. Neonatal results were provided for 284 pregnancies. The reported sensitivity and specificity were 100% and 97.5%, respectively. Diagnostic accuracy was 96.1% including 9/284 inconclusive results. The low number of early gestational age samples is a weakness of the study, and the authors attribute a false negative result to this. The authors concluded that cfDNA for RhD genotyping is an accurate and reliable tool for fetal immunoprophylaxis.

Saramago et al. (2018) conducted an HTA of the use of cfDNA to determine fetal RhD status. The authors searched MEDLINE and other databases, from inception to February 2016, for studies of high-throughput NIPT free-cell fetal deoxyribonucleic acid (DNA) tests of maternal plasma to determine fetal RhD status in RhD-negative pregnant women who were not known to be sensitized to the RhD antigen. Inclusion criteria for all reviews included pregnant women who were RhD negative and not known to be sensitized to the RhD antigen. For examining diagnostic accuracy, inclusion criteria were prospective cohort studies reporting absolute numbers, and for clinical effectiveness, studies that used high throughput NIPT in which anti-D prophylaxis was given and clinical outcomes were reported. The inclusion criteria for implementation outcomes were any publication that reported issues related to the implementation of, or practical advice, relating to NIPT. Eight studies were included in the diagnostic accuracy review, seven studies were included in the clinical effectiveness review and 12 studies were included in the review of implementation. The meta-analysis found that women in the studies were at least 11 weeks gestation or later, and mostly Caucasian with singleton pregnancies. The false negative rate (at risk of sensitization) was 0.34%, and the false positive rate (receiving unnecessary anti-D prophylaxis) was 3.86%. Clinical outcome data was limited to confirm the true sensitization rate, but there was no evidence of potential adverse effects. The authors concluded that there was limited data on clinical effectiveness of NIPT for fetal RhD status, and more studies were needed for non-Caucasians and multiple gestations.

Single Gene Disorders

The use of cfDNA testing to evaluate single gene disorders is in the early stages of development. The evidence available is limited and contains small sample sizes. Further studies with a larger number of patients are needed to determine the clinical utility of this approach.

In the largest clinical validation of carrier screening with reflex to single gene noninvasive prenatal testing (sgNIPT) in a general population setting to date, Wynn et al. (2023) endeavored to strengthen the evidence supporting the use of single gene cfDNA testing to assess the fetal risk of autosomal recessive conditions. Specifically, clinical performance of carrier screening with reflex to sgNIPT was evaluated for four conditions: cystic fibrosis, spinal muscular atrophy, alpha thalassemias, and beta hemoglobinopathies. Pregnancy outcome records of individuals who had undergone this testing were reviewed and a comparison of neonatal outcomes to the fetal risk predicted by the sgNIPT test was performed. A total of 42,067 pregnant individuals from 811 unique practices throughout 45 states and Puerto Rico underwent carrier screening. Of these, 7538 carriers (17.9%) reflexed to sgNIPT. Fetal/neonatal outcomes were obtained for 528 individuals, including 25 impacted pregnancies. High concordance was found between sgNIPT results and neonatal/fetal

outcomes. The sgNIPT assay was found to have a sensitivity of 96% (95% CI: 79.65%-99.90%), specificity of 95.2% (95% CI: 92.98%-96.92%), average PPV of 50.0% (95% CI:35.23%-64.77%), and NPV of 99.8% (95% CI: 98.84%-99.99%). Overall performance of carrier screening with reflex to sgNIPT was determined to have a sensitivity of 92.4% and a specificity of 99.9%. These are not impacted by partner carrier screening or misattributed paternity, in contrast to the traditional carrier screening workflow, for which sensitivity is 35% and maximum PPV is 25%. Based on the results above, the authors assert that carrier screening with reflex to sgNIPT has good performance in a general population and should be considered as first line testing in many situations, including cases where biological partner sample is not available. They indicate that the test used was able to identify high-risk pregnancies related to autosomal recessive conditions with high sensitivity and specificity and has the potential to increase access to actionable health information. Noted limitations included collection of outcomes that relied on individual and provider reporting; of the over 42,000 individuals initially screened, outcomes were received for 528, including only 25 affected neonates. In addition, the authors of this study had affiliations with a test manufacturer who provided financial support for this study, creating a potential for bias.

Adams et al. (2023) conducted a clinical pilot study seeking to determine utility of single gene non-invasive prenatal screening (NIPS-SGD) in a group of high risk pregnant individuals. The NIPS-SGD panel evaluated for pathogenic variants in 30 genes. Pregnant individuals qualifying for study participation had one or more of the following indications: (1) sperm age ≥ 40 years, (2) nuchal translucency ≥ 3.5 mm, (3) fetal anomaly, or (4) family history of a condition included for assessment in the panel. Participants were offered concurrent diagnostic testing. A total of 228 participants completed NIPS-SGD testing and of these, eight (3.5%) had a positive result. No false positive or negative results were identified in 78 participants who underwent diagnostic testing. Ultimately, 41 of the participating individuals received a molecular diagnosis, but 34 of these (82.9%) were outside the scope of the NIPS-SGD test. Positive results from the NIPS-SGD testing impacted medical management for five individuals. The researchers concluded that NIPS-SGD has the potential to detect prenatal diagnoses earlier, which may lead to better monitoring and focused genetic assessment, but diagnostic testing is still preferred when clinically indicated. Additional high-quality validation studies are needed to establish the value-add for NIPS-SGD before this testing can be implemented broadly.

To assess the performance of carrier screening for cystic fibrosis, hemoglobinopathies and spinal muscular atrophy with reflex single-gene noninvasive prenatal screening (sgNIPS), Hoskovec et al. (2023) conducted a study on an unselected population of 9,151 pregnant individuals in the United States. Screening for carrier status of noted conditions was performed, and 1,669 samples (18.2%) were found to be heterozygous for at least one pathogenic variant and reflexed to sgNIPS. The results of sgNIPS were compared with outcomes identified via parent surveys or provider reports for 201 newborns. Overall, informative results were obtained for 98.7% of pregnancies including either negative carrier report or for those identified as heterozygous for a pathogenic variant, a sgNIPS result. In the subgroup with outcomes information, the NPV of sgNIPS was found to be 99.4% (95% CI = 96.0%-99.9%) and the average PPV of sgNIPS was 48.3% (95% CI = 36.1%-60.1%). A key factor was the use of personalized PPVs which accurately reflected the proportion of impacted pregnancies in each PPV range; all pregnancies where sgNIPS fetal risk was found to be > 9/10 (90% PPV) were indeed affected. The authors concluded that prenatal screening with sgNIPS is an option that can provide accurate fetal risk without a paternal screening test and results can be used for counseling and pregnancy management. The study had limitations, including the low number of outcomes collected and a relatively high rate of no-call results (1.3%), all of whom were heterozygous for a pathogenic variant. In addition, outcomes were determined via newborn screening and not through molecular diagnosis, which is the gold standard. Further research including larger cohorts and more complete collection of outcomes, as well as studies that focus on the impact of carrier screening with sgNIPS on clinical practice are needed.

Young et al. (2020) performed a retrospective analysis for non-invasive prenatal diagnosis (NIPD) single-gene testing for pregnancies at risk for cystic fibrosis (CF), spinal muscular atrophy (SMA) and X-linked Duchenne/Becker muscular dystrophies (DMD/BMD) by utilizing the relative haplotype dosage (RHDO) method. RHDO uses a capture-based targeted enhancement, followed by massive parallel sequencing and analysis by relative haplotype dosage. The requirements for NIPD using RHDO include known family history of disorder, confirmed molecular diagnosis, and necessary reference samples when possible. RHDO allows both paternal and maternal inheritance to be determined by measuring allelic imbalance between two haplotypes in cfDNA with phasing conducted through SNP sequencing; multiple single gene disorders can be performed during the same sequencing run and the same assay can be used for all families at risk for a particular condition which eliminates preliminary work-up prior to pregnancy. RHDO for DMD/BMD was performed in at-risk pregnancies following confirmation of a male fetus by cfDNA and requires maternal haplotype with a male reference sample (previous affected, previous unaffected, other male relative affected, or unaffected maternal grandfather) providing the mutated or normal haplotype. Due to the 12% chance for DMD/BMD gene recombination, RHDO was performed 5'-3' and 3'-5'. For CF/SMA, maternal and paternal haplotypes in addition to a reference child (i.e., previously affected child, an unaffected non-carrier child) or a carrier child if parents had different mutations were used for phasing. From September 2016 to October 2019, 152 at-risk pregnancies were referred to the West Midlands Regional Genetics Laboratory. Follow-up genetic testing was performed for 70 of the 146 pregnancies to date for which a diagnostic result was issued. In all

cases, follow-up testing confirmed the RHDO result, and no discrepancies were reported demonstrating 100% concordance. For an additional 39 cases no postnatal discrepancies have been reported to date. The authors concluded that NIPD by RHDO can be performed clinically for both autosomal recessive and X-linked disorders with a high sensitivity and specificity. However, this study is difficult to generalize to a non-selected population as the families selected needed to have a known family history of the disorder. In addition, this study had a lack of follow up data for many cases. In conclusion, the authors emphasized the importance of prenatal counseling for patients undergoing NIPD for single gene disorders and having access to NIPT for routine aneuploidy screening at 10 weeks of pregnancy. Further testing is needed to validate this method for clinical use.

Zhang et al. (2019) developed and reported their clinical validation experience with a novel method for non-invasive prenatal sequencing for a panel of causative genes for frequently occurring monogenic, dominant disorders. Maternal cfDNA was barcoded and enriched and analyzed by next generation sequencing (NGS) for target regions of 30 genes. Low level fetal variants were then determined by a statistical analysis adjusted for NGS read count and FF. Likely pathogenic and pathogenic variants were confirmed by a secondary amplicon-based cfDNA test. Clinical testing was performed on 422 pregnancies, with or without ultrasound findings. Of these 422 cases, 390 had negative testing and 32 had positive results. Follow-up testing on cases was limited and only included 233 of the 422 original cases. The researchers stated that this study revealed 20 true-positives, 127 true-negatives, zero false-positives and zero false-negatives. A significant limitation of this study was the lack of follow up data for many cases therefore the clinical sensitivity and specificity is limited to only cases with outcomes. The authors concluded that by using this novel NIPT NGS method, a large number of dominant, monogenic disorders can be identified however additional validation studies are needed.

Camunas-Soler et al. (2018) developed a method of noninvasive prenatal diagnosis of inherited single-gene disorders using droplet digital PCR from cfDNA. cfDNA and FF were determined using TaqMan assays which target highly variable SNPs. Next, a ratio of healthy and diseased alleles in maternal plasma was quantified using the Taqman assay to target parental mutations. The study involved enrolling pregnant patients who are carriers of mutations causing autosomal-recessive or X-linked disorders for both single mutations and compound heterozygous mutations. Nine pregnancies at risk for different single-gene disorders including: hemophilia, ornithine transcarbamylase deficiency (OTC), cystic fibrosis, B-thalassemia, mevalonate kinase deficiency, acetylcholine receptor deficiency and DFNB1 nonsyndromic hearing loss were testing in the study. For each specimen, the FF and total cfDNA was measured using ddPCR. Primers were designed to amplify the mutation regions and TaqMan probes labeled with different fluorophores against healthy and mutated alleles. Accurate NIPT, according to the authors, relies on comparing the ratio of mutated and healthy alleles in maternal blood with the ratios expected for a healthy or affected fetus. Two affected and 7 unaffected pregnancies were confirmed by follow-up neonatal testing.

Xiong et al. (2018) conducted a feasibility study on patients of Southeast Asian descent to determine if targeted sequencing and relative mutation dosage can be used to correctly identify maternal beta-thalassemia mutations in cffDNA. Samples were collected from 49 couples at risk to have a child with beta-thalassemia, and genomic DNA was evaluated from the parents, cffDNA and either amniocentesis or CVS. Common *HBB* mutations were targeted using nested PCR. Relative mutation dosage was used to determine if the fetus had the wild type allele or the maternal carrier allele. Forty-eight of the samples were able to be classified using cffDNA (98%). The correct result was obtained in 44 of the 48 cases (91.7%), and there was one false positive and three false negatives. The overall sensitivity was 87.5% and the specificity was 95.8% for the inheritance of the maternal allele.

Twin Zygosity

The evidence is insufficient to support the use of NIPT for the sole purpose of determining twin zygosity due to the lack of prospective studies that establish clinical utility.

In an effort to reveal the association between SNP-based cfDNA zygosity results and provider-reported chorionicity, Wojas et al. (2022) evaluated 59,471 samples from twin pregnancies (median gestational age = 12.0 weeks) in a population-based, retrospective cohort study. Assignment of chorionicity was requested on the requisition form, documented as monochorionic (MC), dichorionic, or “don’t know.” The zygosity results from SNP-cfDNA testing [either monozygotic (MZ) or dizygotic (DZ)] were then compared with the chorionicity assignment. A total of 55,344 samples (93.1%) received a zygosity assignment based on the SNP-cfDNA testing. Of these, 30.1% (n = 16,673) were MZ and 69.9% (n = 38, 671) were DZ. When compared with the provider-reported chorionicity, 5.1% (n = 6283) of the twins that had been reported as MC by providers were reported to be DZ using SNP-cfDNA. Noted limitations include lack of knowledge regarding who completed the requestion forms and whether the information included was accurate. In addition, chorionicity information was provided for only 62.7% of total cases and there was no documentation of the method used to determine chorionicity assignment. The study authors believe that the use of SNP-based zygosity assessment should be considered in combination with sonography to expedite interventions for pregnancies at risk for twin-twin transfusion

syndrome (TTTS), hasten referrals to maternal-fetal medicine specialists, and potentially reduce costs of prenatal care for DZ twins. Further research examining the impact of first trimester zygosity assignment with cfDNA testing on the accuracy of chorionicity assignment in twin pregnancies is recommended. At this time, there is little data addressing the effect of zygosity on pregnancy outcomes; this study provides a base for additional research on this subject.

In the first cohort study of twins in which NIPT-based zygosity was correlated with provider-assigned chorionicity, Jelsema et al. (2019) compared ultrasound assignment of chorionicity with SNP-based NIPT to determine zygosity using a Natera proprietary algorithm. Between October 2017 and May 2018, 4,885 twin samples received SNP-based NIPT zygosity determination and established likelihood of aneuploidy for the pregnancy. Chorionicity was determined for 3,949/4,885 (80.8%) of patients. MC was determined in 553 (11.3%); dichorionicity (DC) in 2,330 (47.7%); “don’t know” for 1,066 (21.8%) and “not Recorded” for 936 (19.2%). SNP based NIPT established that 1,450/4,885 (29.7%) were MZ twins and 2,435/4,885 (70.3%) were DZ. Of the “don’t Know” or unrecorded cases (41%), 30.7% were determined to be MZ. Of the twins identified by their provider as MC, 3.4% were found to be DZ and of the DC twins identified by their provider, 12.9% were determined to be MZ. It was reported by the authors that as approximately half of the unspecified/unreported chorionicity samples were determined to be MZ, early and accurate assignment is essential for risk assessment for adverse pregnancy outcome. For the 3.4% of MC determined to be DZ, significant cost reduction TTTS monitoring and maternal anxiety reduction was achieved. With ultrasound findings, accurate NIPT determination allows for better risk counseling for the possibility of one vs. both fetuses having a genetic disorder. SNP-based NIPT can also ensure that experienced providers evaluate MZ twins as early in pregnancy as possible for chorionicity. The authors recommended prospective studies to establish the clinical utility of SNP-based NIPT in twins with assignment of both zygosity and aneuploidy risk.

Vanishing Twin

There is a lack of evidence to support the clinical utility of cfDNA testing in pregnancies with a vanishing twin. Additional studies validating the performance and optimal timing of cfDNA testing in vanishing twin pregnancies is needed.

In a systematic review, van Eekhout et al. (2023) sought to assess the screening performance of NIPT in vanishing twin pregnancies. Literature search was conducted to identify studies addressing test performance of NIPT for trisomy 13, 18, 21, sex chromosomes, and additional findings in pregnancies with VT, through October 2022. The screen positive rate of pooled data and pooled PPV were analyzed using a random effects model. In all, seven studies were included; cohort sizes ranged from five to 767. Pooled data for trisomy 21 showed a screen positive rate of 2.2% (35/1592) with a PPV of 20% (confirmed in 7/35 cases [95% CI 9.8%-36%]). Screen positive rate for trisomy 18 was 0.91% (13/1592) with pooled PPV of 25% (95% CI 1.3%-90%).For trisomy 13, the screen positive rate was 0.44% (7/1592) and confirmation for 0/7 cases (pooled PPV 0% [95% CI 0%-100%]). For additional VT findings, the screen positive rate was 23/767 or 2.9%; none of these could be confirmed. There were no reports of discordant negative results. Based on these results, there is insufficient evidence for evaluation of the use of NIPT in pregnancies with VT. Only seven studies with limited sample sizes and low volumes of aneuploidies per study were identified and included. Meta-analysis was not possible due to the heterogeneity of the study populations and none of the studies included information regarding gestational age. Only one study noted the two types of VT, and the majority of the studies included a high-risk population, so risk of bias was high. The included studies suggested that NIPT detection of common autosomal aneuploidies in pregnancies impacted by VT has been successful, but the false positive rate is higher, likely due to the identification of cfDNA from an aneuploid demised twin. Additional, high quality studies evaluating the performance of NIPT in VT pregnancies and the optimal timing of NIPT are needed. Studies by Pavanello et al. (2021) and Pooh et al. (2021), discussed below in evidence, were included in this systematic review.

Vanadis®

Studies that demonstrate the clinical validity of using the Vanadis NIPT system for determination of chromosome aneuploidy are lacking. Additional studies for clinical validity need to be undertaken prior to acceptance of this screening.

Vanadis NIPT is a different approach to NIPT/NIPS that does not include polymerase chain reaction (PCR) amplification or sequencing (Revvity, 2024). The maternal sample is first treated with a series of enzymes and the targeted cfDNA fragments are captured and converted to DNA circles called rolling circle replication products (RCPs). The RCPs are then converted to fluorescent DNA molecules and labeled with chromosome specific fluorophores. The labeled fluorescent DNA molecules are deposited to a microfilter plate and counted with an automated imaging device. The ratio between the number of each chromosome-specific fluorescent DNA molecules is transferred for risk calculation to proprietary software to calculate the likelihood of a trisomy.

In a recent study, Saidel et al. (2023) used the Vanadis system to screen for trisomies 13, 18, and 21 in pregnant women, comparing positive results to clinical outcomes when those outcomes were available. A total of 8160 samples were

collected from individuals receiving care in a 90-location practice (Women's Health Connecticut). To be included, gestational age of the fetus was required to be at least ten weeks. Thirty-eight individuals were excluded from the study: 32 for gestational age and six for no-call results. The first pass no call rate was 0.98% (80 samples) but 92.5% of these resolved with a second run, for a final no call rate of 0.07%. In total, 8122 specimens were tested with Vanadis and included in the study results. The mean age of participants was 31 years (5867 individuals under the age of 35 and 2255 individuals 35 years or older). The mean gestational age was 12 weeks, 1 day. Of the 92 results that came back screen-positive, 75 (81.5%) had a clinical outcome available and 17 did not. Those screen-positive samples with unavailable outcomes were used only in screen-positive rate calculations and not in the other performance metrics. Forty-three of the 75 screen-positive samples with available clinical outcomes were clinically affected and 32 were not clinically affected. The researchers calculated overall sensitivity and specificity of the Vanadis system at 98% and 99%, respectively, based on available outcomes, and asserted that the Vanadis system met or exceeded the performance of other prenatal testing methods and was comparable to the reported performance of several NGS-based methodologies for cfDNA screening for trisomies 13 and 18. For trisomy 21, three postnatally reported false negative cases led to a lower sensitivity point estimate, but after investigation by the researchers, it was determined that two of the three false negative cases were likely due to a biological limitation that would not have been identified regardless of platform. In this study, the clinical outcomes of completed pregnancies relied on reporting from newborn evaluation, which is in the chart of the newborn and not the birthing parent; therefore, all screen-negative results were not confirmed, which presents a limitation. The authors contend that the Vanadis platform offers a lower cost and has excellent performance with a better no-call rate when compared to another commercial NGS-based NIPT system.

A prospective single-center study led by Conotte et al. (2022) compared the performance of Vanadis with the performance of the Harmony prenatal test. A total of 936 individuals received testing with both tests but participants received the results of the Harmony test only. Either birth outcome or the results of invasive diagnostic tests were used to confirm aneuploidy status for all individuals included in the final assessment. Performance in screening for trisomy 13, 18, and 21 were evaluated by total failure rate. Thirty-six individuals were excluded, leaving a final population of 900 individuals for analysis. The median maternal age was 31 years and maternal weight was 69 kg, with median gestational age at time of testing of 13.3 weeks. The Harmony test identified 34 of 35 cases of trisomy 21 and failed in one case (quality issue). The Vanadis test identified all 35 cases. Eleven of 15 cases of trisomy 18 were identified by Harmony, while Vanadis identified 14 of the 15 cases. Both tests identified three cases of trisomy 13. Overall, after first attempt, Harmony failed in 3.2% of cases (n = 29) and Vanadis failed in 0.2% (2 cases, $p < .05$). Ten of the 29 Harmony failures were due to low FF. High density of spot counts per image led to the two failures of Vanadis. The researchers concluded that this preliminary data indicates that Vanadis performs well in screening for trisomies 13, 18, and 21 and has a low failure rate. They recommend further investigation of this test when FF is under 4%.

Palomaki et al. (2022) conducted a prospective observational cohort study examining a non-sequencing, non-PCR-based methodology using rolling circle amplification (RCA) that is intended to perform as well as conventional NGS screening. The study goal was to evaluate this new methodology by testing samples from pregnancies at 10 to 20 weeks gestation with known outcomes. The primary outcomes goals were to measure Down syndrome DR, Down syndrome false positive rate and Down syndrome failure rate. Secondary outcome measures included Trisomy 18 DR, Trisomy 13 DR, Trisomy 18 false positive rate, Trisomy 13 failure rate, and fetal sex DR. The study population included a low-risk group that consisted of 2,213 pregnancies with no high risk findings (e.g., abnormal ultrasound, positive serum screen) who were undergoing initial clinical cfDNA screening. To simulate a general pregnancy population, approximately 20% of these women were 35 and older. An estimated 2% (48) of these low-risk women had a failed/no call cfDNA test. The high-risk group consisted of 137 women with a positive cfDNA screen reported by a Clinical Laboratory Improvement Amendments (CLIA)-approved commercial laboratory, and who presented for consideration of a confirmatory diagnostic test, (i.e., CVS or amniocentesis). Results were as follows: DR for the common trisomies was 95.9% (117/122, 95% CI, 90.5%-98.5%); overall FPR was 1.00% (22/2,205, 0.65%-1.51%). Test failure rate after repeat testing was 0.04%. When assay SDs were below pre-specified levels, the overall FPR was much lower at 0.30% ($p < 0.001$). Fetal sex calls were correct for 99.7%. One technician analyzed 560 samples over two weeks, a rate of 14,000/year. Of note, no false-negative cfDNA tests were enrolled in the study, which could result in the overestimation of RCA DRs. The authors also recommend focus on higher precision and reduction of collection tubes from two to one. While the results are encouraging, future studies are needed.

In a 2021 observational prospective clinical validation study conducted in Osaka, Japan, Pooh et al. investigated the accuracy of the Vanadis® NIPT test (referred to as CRITO-NIPT for this study) to gain insight into reasons for discrepancies. Testing using CRITO-NIPT was used in 1208 individuals undergoing CVS or amniocentesis after detailed fetal ultrasound and genetic counseling. Results of CRITO-NIPT were compared with invasive genetic testing results. If test discrepancies were found, materials from placentae were collected for further genetic research and use of pre-procedure fetal ultrasound. The researchers found the positive predictive value of CRITO-NIPT for trisomy 21 was 93.55%, trisomy 18 was 88.46% and trisomy 13 was 100%. Placentae were examined in 90% of false positive cases and in 75% of the CRITO-false positive trisomy 21 cases, placental mosaicism or a demised twin's trisomy 21 were confirmed.

Complex mosaic cases were also found, including tetrasomy21/trisomy7 and monosomy 21/trisomy21. The authors note the potential of rolling circle replication as a powerful new platform and place focus on the importance of the use of the detailed fetal ultrasound, but also highlight the ethical concerns raised by NIPT testing. Of note, all study participants were high-risk, making the results of the study difficult to generalize to a larger population. In addition, three authors in this study were reported to have potential conflicts of interest.

Pavanello et al. (2021) conducted a prospective study among women who had been referred for invasive prenatal diagnosis to assess efficacy of cfDNA screening for aneuploidy using the rolling circle replication system. The study included 805 women (27 with twin pregnancy) considered to have high risk pregnancies. During the study, researchers noticed that the quality of analytic runs was decreased systematically and after investigation, found that the room temperature in the summer months exceeded the requirements of the instruments. To address this issue, replacements to the instruments were made and additional air conditioning was installed. Unfortunately, the problem persisted and the Vanadis system was subsequently moved to a different laboratory space. Screening performance was separately assessed in the runs prior to and after relocation of the system. Results of this study indicate that in singleton pregnancies, the down syndrome DR was 100% and FPR was 0.14%. Edwards syndrome DR was 96% with FPR of 0.78% and Patau syndrome DR was 67% with FPR of 0.26%. Overall, the study found 48 cases of Down syndrome, 25 cases of Edwards syndrome and 3 cases of Patau syndrome. The no-call rate was 2.6%. This comparatively high rate was attributed to the decreased quality of assay runs in the early part of the study related to the room temperature issues, as the no-call rate after the equipment was moved to a cooler space decreased from 4.7% to 1.1%. Likewise, all the false positive results in the study were obtained prior to the equipment move as well. The authors concluded that cfDNA rolling circle method can yield similar results to other cfDNA methods when room temperature is adequately controlled, however the temperature and equipment issues bring into question the accuracy of the data. In addition, since the study was conducted on population of individuals with high-risk pregnancies, the results are not necessarily generalizable to a broader population.

Gormus et al. (2021) published a validation study from clinical laboratories using the rolling circle replication (RCR) technology. Testing was performed on 831 samples from spontaneously pregnant women (singleton pregnancy) and 25 synthetic samples. The women were not selected based on prior risk. Risk of trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) were analyzed by three separate laboratories on three continents. Any individuals with positive screening results were provided confirmatory invasive diagnostic testing and genetic counseling. Individuals with negative screening results were evaluated for fetal aneuploidy at birth using newborn evaluations and assessments. The researchers assert that their study found RCR to be highly viable for aneuploidy assessment with 100% sensitivity for T21 (95% CI: 82.35-100.00%), 100% sensitivity for T18 (71.51-100.00%), and 100% sensitivity for T13 (66.37-100.00%). No false negative results were detected, and levels of false positive rates were low (FPR: 0.24% for T21, 0.47% for T18, and 0.24% for T13). First-pass no-call rate was 0.93%. Results were comparable to the more common NIPT technologies using NGS. The authors concluded that the high sensitivity and specificity of Vanadis NIPT make it an efficient and cost-effective option for NIPT. Of note, all of the study authors are current or former employees of PerkinElmer, Inc, the manufacturer of the Vanadis® NIPT system.

Ericsson et al. (2019) performed a clinical validation study to evaluate the clinical performance of a new automated cfDNA assay in maternal plasma screening for trisomies 21, 18, and 13, and to determine fetal sex. Plasma samples from 1200 singleton pregnancies, from prospective or retrospective cohorts, were analyzed with this new non-sequencing cfDNA method. The method uses direct quantification of targeted chromosomal fragments labelled by rolling circle replication. The results were compared to the reference outcomes by cytogenetic testing, of amniotic fluid or chorionic villi, or clinical examination of neonates. The samples examined included 158 fetal aneuploidies. Sensitivity was 100% (112/112) for trisomy 21, 89% (32/36) for trisomy 18, and 100% (10/10) for trisomy 13. The respective specificities were 100%, 99.5%, and 99.9%. There were five first pass failures (0.4%), all in unaffected pregnancies. The authors concluded that the new automated cfDNA assay has high sensitivity and specificity for trisomies 21, 18, and 13 and accurate classification of fetal sex, while maintaining a low failure rate. Limitations of the study include study design, study size, and use of an enriched cohort with high risk and known affected pregnancies.

Dahl et al. (2018) reported a proof-of-concept study on a new approach to NIPT testing that uses novel molecular probe technology to label target chromosomes, then uses a nanofilter to enrich single molecules for imaging and counting. This allows for identification of aneuploidy without the need for DNA amplification, microarrays or sequencing. The authors developed probes that were designed to capture and label rolling circle replication products (RCPs) from 3500 loci on chromosome 21, as well as an optically transparent nanofilter 96-well plate to capture the RCPs. Four enzymatic processes result in labelled RCPs. First, the target chromosomes are broken up into cfDNA targets, and then the fragments are mixed with, and hybridized to, a complementary probe set that allows for chromosome specific labelling. The probes are designed so that when perfect hybridization occurs, a circle is formed. Next, exonucleases are used to remove all remaining linear DNA and unused probes. In the final step, primers are added, and DNA circles are combined

with polymerases, so the circles are copied to a clonal concatenated RCP. Each original cfDNA target fragment generates one clonally amplified RCP that collapses in solution into a sub-micron sized DNA bundle. The RCPs are labelled with fluorescent nucleotides, and then added to the nanfilter-96 well plate. The labelled RCPs are imaged through the bottom of the plate using the Vanadis plate scanner, which quantifies the labelled RCPs. The authors used data from a known dataset of 10,698 pregnancies from gestational weeks 10-14 to determine the precision of the assay to identify the different FF levels to eliminate to achieve the maximal DR and low false positive rate. The specificity of probe capture was determined using cell lines and was > 99%, and reference samples with pre-determined amounts of trisomy DNA were used to identify the FF measurable. FF at 4% or higher were clearly identified. Proof of concept on clinical samples was performed on a blinded set of 17 trisomy 21 samples in a cohort of 165 normal samples. Using an age adjusted risk cutoff of 1% risk, all affected and normal samples correctly identified. A prospective, high risk singleton pregnancy sample cohort of 104 women was also tested. All patients were examined by ultrasound to record the gestational age by measurement of the crown-rump length, to diagnose major fetal abnormalities and to measure NT thickness. Maternal serum levels of pregnancy associated plasma protein (PAPP)-A and free β-chorionic gonadotropin (hCG) were determined. Thirteen trisomy 21 pregnancies were correctly identified, and no false positives were found.

Clinical Practice Guidelines

American College of Medical Genetics and Genomics (ACMG)

AMCG published a guideline (Dungan et al., 2023) addressing the use of prenatal cfDNA screening for fetal chromosome abnormalities in general-risk populations. The guideline was largely based on the results of the 2022 ACMG systematic review (Rose et al., discussed above) and used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Evidence to Decision framework to establish recommendations. The evidence reliably demonstrated better accuracy of prenatal cfDNA screening in comparison with traditional screening techniques for trisomies 13, 18, and 21 in both singleton and twin gestation pregnancies and noted that the identification of rare autosomal trisomies and other microdeletion syndromes with prenatal cfDNA screening is “an emerging area of interest.” Specific recommendations are as follows:

- Prenatal cfDNA screening for fetal trisomies 13, 18, and 21 is recommended over traditional screening methods for all pregnant patients with singleton gestation pregnancies. (Strong recommendation based on high certainty of evidence)
- Prenatal cfDNA screening for fetal trisomies is recommended over traditional screening methods for twin gestation pregnancies. (Strong recommendation based on high certainty of evidence)
- Prenatal cfDNA screening should be offered to individuals with single gestation pregnancies to assess for fetal SCA. (Strong recommendation, based on high certainty of evidence)
- ACMG suggests offering prenatal cfDNA screening for 22q11.2 deletion syndrome to all pregnant individuals. (Conditional recommendation, based on moderate certainty of evidence)
- Currently, there is insufficient evidence for the recommendation of routine screening for CNVs other than 22q11.2 deletions. (No recommendation, owing to lack of clinically relevant evidence and validation)
- Currently, there is insufficient evidence to either recommend, or not recommend, prenatal cfDNA screening for the identification of RATS. (No recommendation, owing to lack of clinically relevant evidence)

In addition to the above recommendations, the ACMG guideline indicates that the most frequent explanation for no-call results in prenatal cfDNA screening is insufficient FF. Low FF has been linked to varying adverse pregnancy outcomes, but definitive rates of pregnancy complications and standard monitoring practices have not been determined. ACMG also notes that certain pregnancy factors can interfere with the performance of prenatal cfDNA screening; vanishing twin syndrome is known example.

American College of Obstetricians and Gynecologists (ACOG)

ACOG’s NIPT Summary of Recommendations includes the following:

- Prenatal genetic screening options including serum screening with or without nuchal translucency ultrasound or cfDNA screening and diagnostic testing with chorionic villus sampling (CVS) or amniocentesis should be discussed and offered to all pregnant individuals regardless of age or risk of chromosomal abnormality.
- If screening is accepted, individuals should have one prenatal screening approach; multiple screening tests should not be performed simultaneously.
- cfDNA is the most sensitive and specific screening test for the most common fetal aneuploidies. However, the potential for false-positive and false-negative results exists. Importantly, cfDNA testing is not equivalent to diagnostic testing.
- Individuals whose screening tests are positive for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with opportunity for further diagnostic testing to confirm results.

- Individuals whose screening tests are negative should be informed that although this result substantially decreases their risk of the targeted aneuploidy, it does not ensure that the fetus is unaffected. Other genetic disorders that are not part of the screening/testing should be reviewed.
- In cases of cfDNA screening test results that are not reported by the laboratory or are uninterpretable (a no-call test result), individuals should be informed that test failure is associated with an increased risk of aneuploidy. They should undergo further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.
- cfDNA screening used as follow-up for individuals with a screen positive serum analyte screening test result is an option for individuals who want to avoid invasive diagnostic testing. Individuals must be informed that this approach could delay a definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.
- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be part of pretest counseling performed for individuals with multiple gestations. Overall, performance of screening for trisomy 21 by cfDNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. As such, it is difficult to determine an accurate DR for trisomy 18 and 13.
- Prenatal screening and prenatal diagnosis should be offered to all individuals regardless of previous preimplantation genetic testing, as preimplantation genetic testing is not uniformly accurate.
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, a significant risk of an inaccurate test result exists when serum-based aneuploidy screening or fetal cfDNA screening is used. In these cases, individuals should be counseled, and diagnostic testing should be offered.
- When unusual or multiple aneuploidies are detected by cfDNA, affected individuals should be referred for genetic counseling and maternal-fetal medicine consultation. (ACOG, 2024).

An ACOG practice advisory recognizes the emerging technology and availability of cfDNA screening for single-gene disorders but emphasized that there is insufficient evidence to demonstrate accuracy and positive and negative predictive values for general population use (ACOG, 2019; reaffirmed 2023). For this reason, ACOG does not recommend single gene cfDNA screening in pregnancy.

American College of Obstetricians and Gynecologists (ACOG)/Society for Maternal-Fetal Medicine (SMFM)

Pregnancy at 35 years of age or older was the focus of Obstetric Care Consensus number 11, developed jointly by ACOG and SMFM (2023). In this consensus ACOG and SMFM recommend that prenatal genetic screening options (serum screening with or without nuchal translucency ultrasound or cfDNA screening) as well as diagnostic testing (chorionic villus sampling or amniocentesis) be discussed and offered to all pregnant individuals regardless of their risk of chromosomal abnormality or age. Each individual has the right to either pursue or decline genetic screening and diagnostic testing. (GRADE 1A; strong recommendation, high-quality evidence).

In Practice Bulletin 231, ACOG and SMFM (2021) address prenatal screening for fetal chromosome abnormalities in multi fetal gestation pregnancies indicating that all women with multifetal gestations, regardless of age, are candidates for screening for fetal chromosome abnormalities, however, no method of fetal chromosome abnormality screening including serum samples will be as accurate in twin gestations as it is in singleton gestations. The bulletin asserts that it is important to include this information in the counseling process for patients with multiple gestation pregnancies. No data exists for serum screening for high-order multiple gestations (e.g., triplets or quadruplets). Analyzing risk vs. benefit for screening/diagnostic testing in individuals carrying multiple fetuses is complex. cfDNA screening can be performed in twin pregnancies, however because there is a smaller number of reported affected cases than in singleton pregnancies, it is challenging to determine accurate DR for trisomy 18 and 13. Since twin fetuses in a single pregnancy each contribute variable amounts of cfDNA, it's possible that a fetus with a chromosomal abnormality would contribute less fetal DNA, thereby masking the aneuploid test result. Though recent studies have suggested the sensitivity for trisomy 21 with cfDNA in twin pregnancies may be similar to singletons, there has been a higher rate of test failure in twins.

ACOG and SMFM addressed screening for fetal chromosomal abnormalities in ACOG Practice Bulletin Number 226 (2020).

Level A recommendations (based on good and consistent scientific evidence) regarding cfDNA include:
- Prenatal genetic screening (serum screening with or without nuchal translucency ultrasound or cfDNA screening) and diagnostic testing should be offered to all pregnant women regardless of maternal age or risk for chromosome abnormality.
- If screening is accepted, patients should only have one screening performed and not multiple screening tests performed simultaneously.

- cfDNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cfDNA testing is not equivalent to diagnostic testing.
- Patients with positive screening should have genetic counseling, comprehensive ultrasound and be offered diagnostic testing.
- Patients whose cfDNA are not reportable are at increased risk for chromosomal aneuploidy and should be offered genetic counseling, comprehensive ultrasound and diagnostic testing.

Level B recommendations (based on limited or inconsistent scientific evidence) regarding cfDNA include:
- The use of cfDNA as follow-up for patients with a screen positive serum-analyte test results is an option for patients who want to avoid diagnostic testing.
- In situations of isolated, soft ultrasound markers and no prior screening has been performed cfDNA, quad screen or diagnostic testing should be offered.
- cfDNA screening can be performed for twin pregnancies. Overall performance of screening for trisomy 21 by cfDNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate DR for trisomy 18 and 13.
- Prenatal screening and prenatal diagnosis should be offered to all pregnant individuals regardless of previous preimplantation genetic testing.

Level C recommendations (primarily based on consensus/expert opinion) regarding cfDNA include:
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cfDNA is used. In these situations, the pregnant individual should be counseled on this information and offered diagnostic testing.
- Patients with unusual or multiple aneuploidies detected by cfDNA should be referred for genetic counseling.

In addition, it is noted in this bulletin that although screening for a limited number of microdeletions with cfDNA is available, this testing has not been clinically validated and is not recommended. With regard to CNV testing, the practice bulletin states there is currently no genetic screening test available to comprehensively screen for all CNVs. Another type of testing offered by some laboratories includes genome-wide cfDNA screening for large deletions and duplications, analyzing the whole genome to potentially detect abnormalities larger than what cfDNA microdeletion screening can accomplish. This type of testing is not recommended by ACOG/SMFM as it has not been clinically validated and accuracy of detection/false-positive rates have not been determined. Lastly, the bulletin notes that “although repeat screening may be considered in the setting of a sample drawn at an early gestational age or a specific concern regarding sample characteristics, because repeat sampling delays a diagnostic test, it is not advised if screening results are consistent with sonographic anomalies, or if a patient is at a gestational age at which the delay may compromise their reproductive options.”

In Practice Bulletin 181, ACOG notes that while there is improved accuracy of noninvasive fetal RhD genotyping, comparisons with current routine prophylaxis of anti-D immunoglobulin at 28 weeks of gestation have not shown a consistent benefit, and noninvasive assessment of fetal RhD status is not recommended for routine use at this time (ACOG, 2017; reaffirmed 2019).

American Society of Human Genetics (ASHG)/European Society of Human Genetics (ESHG)

In a joint statement, ASHG and ESHG present different scenarios for NIPT-based screening for common autosomal aneuploidies. The statement suggests that trade-offs involved in these scenarios should be assessed in light of the aim of screening, the balance of benefits and burdens for pregnant women and their partners. The statement includes the following recommendations:
- NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as combined first-trimester screening. However, a positive NIPT result should not be regarded as a final diagnosis: false positives occur for a variety of reasons. Women should be advised to have a positive result confirmed through diagnostic testing if they are considering a possible termination of pregnancy.
- Expanding NIPT-based prenatal screening to also report on sex chromosomal abnormalities and microdeletions is not recommended.

When the test is assessed in terms of its predictive value, the low prevalence of the relevant conditions in the target population must be taken into consideration. For instance, the PPV in a general risk population can result in a higher rate of false alarms because of the lower prevalence of aneuploidies in this population (Dondorp et al., 2015).

International Society for Prenatal Diagnosis (ISPD)

To update information regarding current technologies, implementation practices and clinical experiences, the ISPD published a new position statement on the use of non-invasive prenatal testing for fetal chromosomal conditions in 2023. The consensus position offered by the ISPD is summarized as follows:

- NIPT is the most accurate screening test for the common autosomal aneuploidies (trisomies 21, 13 and 18) in unselected singleton populations, and those at known increased probability. It can be offered in primary or contingent screening models with context-specific considerations in local health policy influencing decisions and implementation models.
- False-positive results occur with NIPT. Therefore, ISPD strongly recommends that all patients with a high chance of NIPT result have genetic counseling and are offered diagnostic testing, particularly if the termination of pregnancy is being considered.
- Fetal fraction is an important quality control metric, but substantial variation exists between laboratories and test methodologies. Laboratories should perform their own internal validation of their limit of detection and threshold for 'no call' results.
- Providers (laboratory and clinicians) should have established clinical pathways for the management of patients with a "no call" result. This may include detailed ultrasound, offer of repeat NIPT, alternative screening test, and/or diagnostic testing.
- If technically relevant, protocols for the identification and disclosure of suspected malignancy should be developed by laboratories.
- NIPT for SCA is sufficiently accurate to be offered alongside autosomal aneuploidy screening with specific pretest counseling and consent. However, other societal, economic, cultural and ethical factors may need to be considered in health policy decisions regarding population-based screening for the sex chromosomes.
- There is insufficient data to assess the performance and clinical utility of routine NIPT for rare autosomal trisomies, sub-chromosomal imbalances and microdeletion/duplication syndromes. Further research is required to evaluate these applications of NIPT, but if offered as part of local practice there should be protocols in place to manage high-risk results and detailed platform-specific counseling available both pre- and post-testing.
- At least one early first trimester scan for dating, diagnosis of multiple pregnancy, and confirmation of fetal viability should be offered before performing NIPT.
- Fetuses with ultrasound abnormalities, including NT measurement ≥ 3.5 mm, should be offered diagnostic testing and evaluation with chromosomal microarray regardless of the prior NIPT result.
- The ethical implementation of NIPT requires attention to provision of quality pre-testing counseling, equity of access, and access to appropriate downstream clinical services.
- All stakeholders, including healthcare consumers, should be involved in determining local implementation models and future directions for NIPT. (Hui et al., 2023).

The ISPD addresses the use of cfDNA screening for Down syndrome in multiple gestation pregnancies in a 2021 position statement (Palomaki et al.). They assert that although only 3/10 professional society statements allow or recommend cfDNA screening in twin pregnancies, cfDNA screening for common autosomal trisomies in twin pregnancies is appropriate due to sufficient evidence showing high detection and low false positive rates. They further recommend counseling and offer of diagnostic testing for confirmation if the cfDNA screening reveals increased risk.

National Society of Genetic Counselors (NSGC)

In a 2021 position statement, the NSGC states its belief that all pregnant individuals, regardless of aneuploidy risk, should have access to prenatal screening using cfDNA. They recommend that healthcare providers present cfDNA for aneuploidy as a topic within the context of other prenatal screening and testing options, including the option of pursuing diagnostic testing as a first-line approach or declining any screening or testing altogether. Discussions should also include individual preferences, values and needs as well as the limitations and the benefits of genetic screening with cfDNA. They further recommend careful consideration of the test's PPV, particularly in rare disorders.

U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

Laboratories that perform DNA-based prenatal tests for trisomy 21, 18, and 13 are regulated by the FDA under the Clinical Laboratory Improvement Amendments. Refer to the following website for more information: https://www.fda.gov/medical-devices/ivd-regulatory-assistance/clinical-laboratory-improvement-amendments-clia. (Accessed February 7, 2024)

Additional Product Information (Not All Inclusive)

- Harmony™ Prenatal Test (Roche)
- MaterniT21® PLUS (LabCorp®)
- Panorama™ Prenatal Test (Natera™ Inc.)
- PreSeek™ (Baylor Genetics)
- QNatal® Advanced (Quest Diagnostics™)
- SensiGene (Sequenom Laboratories)
- UNITY Screen™ (Billion to One)
- Vanadis™ NIPT Test (Revvity)
- Verifi® Prenatal Test (Illumina®, Inc.)
- Vistara™ (Natera™ Inc.)

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Policy History/Revision Information

Date	Summary of Changes

09/01/2024	**Coverage Rationale**
	• Replaced language indicating "DNA-based noninvasive prenatal tests are unproven and not medically necessary for *any* of the [listed indications]" with "DNA-based noninvasive prenatal tests are unproven and not medically necessary *for all other indications [not listed as proven and medically necessary in the policy] including* [the listed indications]"
	• Revised list of unproven and not medically necessary indications:
	  ○ Added "genome-wide or exome-wide screening (e.g., MaterniT Genome)"
	  ○ Replaced:
	  ▪ "Screening for microdeletions" with "screening for microdeletions/*microduplications/copy number variations (CNVs)*"
	  ▪ "Fetal RhD status" with fetal RhD *or fetal antigen* status"
	• Updated list of examples of screenings for single gene disorders; removed "Unity Carrier Testing™"
	Applicable Codes
	• Removed CPT code 0449U
	• Added ICD-10 diagnosis code Z36.9
	• Removed ICD-10 diagnosis code O35.1XX0
	• Revised description for ICD-10 diagnosis codes Z34.00, Z34.01, Z34.02, and Z34.03

Date	Summary of Changes
	• Removed notation indicating CPT code 0060U is not on the State of New Jersey Medicaid Fee Schedule and therefore are may not be covered by the State of New Jersey Medicaid Program **Supporting Information** • Updated *Description of Services*, *Clinical Evidence*, *FDA*, and *References* sections to reflect the most current information • Archived previous policy version CS085NJ.X

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the federal, state or contractual requirements for benefit plan coverage must be referenced as the terms of the federal, state or contractual requirements for benefit plan coverage may differ from the standard benefit plan. In the event of a conflict, the federal, state or contractual requirements for benefit plan coverage govern. Before using this policy, please check the federal, state or contractual requirements for benefit plan coverage. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

UnitedHealthcare may also use tools developed by third parties, such as the InterQual® criteria, to assist us in administering health benefits. The UnitedHealthcare Medical Policies are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.