Cell-Free Fetal DNA Testing

Policy Number: CS085.AA
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Instructions for Use

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Related Community Plan Policies

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Application

This Medical Policy does not apply to the states listed below; refer to the state-specific policy/guideline, if noted:

State	Policy/Guideline
Indiana	None
Kentucky	Cell-Free Fetal DNA Testing (for Kentucky Only)
Louisiana	Cell-Free Fetal DNA Testing (for Louisiana Only)
Mississippi	None
Nebraska	Cell-Free Fetal DNA Testing (for Nebraska Only)
New Jersey	Cell-Free Fetal DNA Testing (for New Jersey Only)
North Carolina	Cell-Free Fetal DNA Testing (for North Carolina Only)
Ohio	Cell-Free Fetal DNA Testing (for Ohio Only)
Pennsylvania	None
Tennessee	Cell-Free Fetal DNA Testing (for Tennessee Only)

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 any of the following:

- 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
- Conditions including, but not limited to, the following:
  - Pregnancies involving one or more of the following:
    - Three or more fetuses
    - Fetal demise in a multiple gestation pregnancy
    - Vanishing twin syndrome
  - Repeat testing due to low fetal fraction
  - Missed abortion/fetal demise in a single gestation pregnancy
  - Screening for the following:
    - Aneuploidy other than Trisomies 21, 18, 13 or sex chromosomes
    - Microdeletions
    - Single gene disorders (e.g., Vistara™, PreSeek™, Unity™ Carrier Testing)
    - Fetal RhD 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, 2023].

Cell Free Fetal DNA (cffDNA or cfDNA): Small fragments of fetal DNA from the placenta that moves freely in the pregnant individual's blood. These fragments can be analyzed via a noninvasive prenatal screening test. (ACOG Dictionary, 2023).

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, 2022).

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 cell-free fetal DNA (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, 2022).

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
0449U	Carrier screening for severe inherited conditions (e.g., cystic fibrosis, spinal muscular atrophy, beta hemoglobinopathies [including sickle cell disease], alpha thalassemia), regardless of race or self-identified ancestry, genomic sequence analysis panel, must include analysis of 5 genes (CFTR, SMN1, HBB, HBA1, HBA2)

CPT Code	Description
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

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
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

Diagnosis Code	Description
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
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

Diagnosis Code	Description
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
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

Diagnosis Code	Description
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
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

Diagnosis Code	Description
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
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

Diagnosis Code	Description
Q95.8	Other balanced rearrangements and structural markers
Q95.9	Balanced rearrangement and structural marker, unspecified
Z34.00	Encounter for supervision of normal pregnancy, unspecified trimester
Z34.01	Encounter for supervision of normal pregnancy, first trimester
Z34.02	Encounter for supervision of normal pregnancy, second trimester
Z34.03	Encounter for supervision of normal 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
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
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

Diagnosis Code	Description
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

Routine screening tests for trisomies 21, 18 and 13 include first-trimester screening (which involves an ultrasound and a blood test), maternal serum screening (a blood test) and a high-resolution ultrasound evaluation in the second trimester. These tests may identify women with an increased risk of having a child with trisomy 21, 18, or 13, 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. During pregnancy, there are cfDNA fragments from both the mother and fetus in maternal circulation. The tests detect the fetal component of chromosomal material in maternal blood and can be offered as early as 9 weeks of pregnancy. Available tests use different methodologies and algorithms for data analysis (ACOG, 2020).

Clinical Evidence

Aneuploidy for Trisomies 21, 18, and 13

In a 2022 ACMG systematic evidence review, Rose et al. evaluated the performance of 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, 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 sex chromosome aneuploidies (≥ 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. Authors, 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 cell-free DNA (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 six 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 three 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.

A recent Hayes report (2021a, updated 2022) 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 false-positive rate (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.

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 positive predictive value (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 sex chromosome aneuploidy were suggested.

The Washington State Health Care Authority commissioned a health technology assessment (HTA) on cell-free fetal DNA from the Center for Evidence-based Policy Oregon Health & Science University, and a final report was issued in December 2019 (Washington State, 2019). The report reviewed available peer reviewed literature and concluded that for all age groups, cell-free fetal DNA testing for aneuploidy has a lower FPR than conventional screening and results in a lower rate of invasive testing than conventional aneuploidy screening.

The Province of Ontario, Canada commissioned a HTA of NIPT in 2019 (Ontario, 2019). The HTA committee reviewed available evidence in peer-reviewed literature and concluded that NIPT “NIPS 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.

The performance of cell-free DNA screening (QNatal advanced) for trisomies 21, 18, 13 and sex chromosome aneuploidies (SCA) 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 cell-free screening. Eighty-seven percent 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 cell-free DNA 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.

Chen et al. (2018) studied the ability for NIPT to detect chromosome aneuploidy in a cohort of 4,194 women undergoing cytogenetic prenatal diagnosis at the Changzhou Maternity and Child Health Care Hospital between January 2009 and October 2017. Patients were over 35 years old and between 17-23 weeks gestation. Cell-free fetal DNA was analyzed using MPSS. Routine karyotyping identified 233 fetuses with chromosome abnormalities, for an overall incidence of 5.6%. There were 132 cases of trisomy 21, of which four were caused by a Robertsonian translocation, 14 were mosaic, and 21 cases had an abnormal chromosome structure. There were 37 trisomy 18, four trisomy 13, five monosomy X, 32 other sex chromosome abnormalities, and two other autosomal aneuploidies. In comparison, NIPT identified 204 of the cases with abnormal chromosomes. The cases missed included one trisomy 21, one trisomy 18, two other autosomal aneuploidies, six chromosome aneuploidy mosaics, and all 21 structurally abnormal chromosomes resulting in trisomy 21. The authors conclude that using NIPT alone will miss 12.4% of fetal chromosome abnormalities if it completely replaces prenatal diagnosis for women of advanced maternal age, but they noted that in this study, the anomalies missed are not associated with advanced maternal age.

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 sex chromosome aneuploidy (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.

Five clinical laboratories in the Netherlands, Australia, Denmark, and Brazil collaborated to review their outcome records for true positive trisomy 21 and false negative cases (Huijsdens-van Amsterdam et al., 2018). The labs represented all major NIPT technologies of massively parallel shotgun sequencing (MPSS), targeted MPS (tMPS), targeted microarray (tMA), and targeted single nucleotide polymorphism (tSNP). Overall, the labs documented 646 confirmed cases of trisomy 21 and nine false negative cases, resulting in a false negative rate of 1.4%. There were four cases missed of classic trisomy 21, and five cases with a de novo 21q:21q rearrangement. Two of the five were confirmed to be isochromosomes. In one case, placental material was available and placental mosaicism was confirmed. The authors noted that a low trisomic fraction relative to the fetal fraction suggests placental mosaicism. The average fetal fraction in 21q:21q cases was 10%. When combined with the literature on false negative trisomy 21 results, 21q:21q rearrangements represented 28% of all false negatives.

Mackie et al. (2017) conducted a meta-analysis of cohort studies reporting on cell-free fetal DNA NIPT 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.

Iwarsson et al. (2017) conducted a systematic review and meta-analysis to assess the performance of cfDNA testing for the detection of trisomy 21, 18, and 13 in a general pregnant population and update the data on high-risk pregnancies. In a general pregnant population, the authors noted moderate evidence of a pooled sensitivity of 0.993 and specificity of 0.999 for the analysis of trisomy 21. Pooled sensitivity and specificity for trisomies 13 and 18 were not calculated in this population due to the low number of studies. In a high-risk pregnant population, the authors noted moderate evidence of pooled sensitivities for trisomies 21 and 18 of 0.998 and 0.977, respectively, and low evidence of a pooled sensitivity for trisomy 13 of 0.975. The pooled specificity for all three trisomies was 0.999. The authors concluded that cfDNA performs well as a screen for trisomy 21 in a general pregnant population; however, more data is needed for trisomies 18 and 13.

Norton et al. (2016) compared the performance of sequential and cfDNA screening for detection of fetal chromosomal abnormalities in a general prenatal cohort. Of 452,901 women who underwent sequential screening, 2,575 (0.57%) had a fetal chromosomal abnormality. The DR of sequential screening for all aneuploidies in the cohort was greater than cfDNA. The authors concluded that cfDNA should not be adopted as a primary screening tool without further evaluation of the implications for detection of all chromosomal abnormalities and how to best evaluate no results cases.

Taylor-Phillips et al. (2016) conducted a systematic review and meta-analysis to measure the accuracy of NIPT for Down, Edwards, and Patau syndromes using cfDNA. Pooled sensitivity was 99.3% for Down, 97.4% for Edwards, and 97.4% for Patau syndrome. The pooled specificity was 99.9% for all three trisomies. Sensitivity was lower in twin than singleton pregnancies, reduced by 9% for Down, 28% for Edwards, and 22% for Patau syndrome. Pooled sensitivity was also lower in the first trimester of pregnancy in studies of the general obstetric population. NIPT using cfDNA has very high sensitivity and specificity for Down syndrome, with slightly lower sensitivity for Edwards and Patau syndrome. NIPT should not be used as a final diagnosis for positive cases.

Zhang et al. (2015) reported the clinical performance of MPS-based NIPT in detecting trisomies 21, 18, and 13 and to compare its performance in low-risk and high-risk pregnancies. NIPT was performed and results obtained in 146,958 samples, for which outcome data were available in 112,669 (76.7%). Repeat blood sampling was required in 3,213 cases and 145 had test failure. Aneuploidy was confirmed in 720/781 cases positive for trisomy 21, 167/218 cases positive for trisomy 18, and 22/67 cases positive for trisomy 13. Nine false negatives were identified, including six cases of trisomy 21 and three of trisomy 18. The overall sensitivity of NIPT was 99.17%, 98.24%, and 100% for trisomies 21, 18, and 13, respectively, and specificity was 99.95%, 99.95%, and 99.96% for trisomies 21, 18, and 13, respectively. In the low-risk population, the PPV was 81.36% for trisomy 21. The reduced PPV in the low-risk group as a consequence of lower disease prevalence, reaffirmed that NIPT should not be used as a diagnostic test and that confirmation by invasive testing is still necessary. The authors reported no significant difference in test performance between 72,382 high-risk and 40,287 low-risk pregnancies (sensitivity, 99.21% vs. 98.97%; specificity, 99.95% vs. 99.95%). The major factors contributing to false-positive and false-negative results were maternal copy number variant and fetal/placental mosaicism. An author noted limitation was the incomplete follow-up of NIPT results, which could introduce bias into the performance evaluation.

In a prospective, multicenter, blinded study, Norton et al. (2015) evaluated cfDNA testing in the assessment of risk for trisomy 21 in a large, routine prenatal screening population. Pregnant women presenting for aneuploidy screening at 10 to 14 weeks of gestation were assigned to undergo both standard screening (with measurement of nuchal translucency and biochemical analytes) and cfDNA testing. The study also evaluated the performance of cfDNA testing and standard screening in the assessment of risk for trisomies 18 and 13. Determination of the birth outcome was based on diagnostic genetic testing or newborn examination. Of 18,955 women who were enrolled, results from 15,841 were available for the primary analysis. The mean maternal age was 30.7 years, and the mean gestational age at testing was 12.5 weeks. Trisomy 21 was detected in 38 of 38 women (100%) in the cfDNA-testing group, as compared with 30 of 38 women (78.9%) in the standard screening group. FPRs were 0.06% in the cfDNA group and 5.4% in the standard-screening group. The PPV for cfDNA testing was 80.9%, as compared with 3.4% for standard screening. In a secondary analysis of 11,994 women with low-risk pregnancies based on a

maternal age < 35, cfDNA testing identified 19 of 19 women with trisomy 21, with six false positive results. Among the 14,957 women for whom standard screening showed a risk of less than 1 in 270, cfDNA testing identified eight of eight women with trisomy 21, with eight false positive results. The PPV for cfDNA testing was 76.0% for women under the age of 35 years and 50.0% for those with a negative result on standard screening. Of the ten cases of trisomy 18 in the primary analysis population, cfDNA testing identified nine and standard screening identified eight. cfDNA testing had a false positive rate of 0.01% and a PPV of 90.0%, as compared to a false positive rate of 0.31% and a PPV of 14.0% with standard screening. Among the 11,185 women who underwent both cfDNA testing and standard screening for trisomy 13, there were two confirmed cases. Of these, cfDNA testing identified two and standard screening identified one. cfDNA testing had a false positive rate of 0.02% compared to 0.25% for standard screening.

Gil et al. (2014) performed a meta-analysis of cfDNA in maternal blood in screening for aneuploidies. Weighted pooled detection rate (DR) and FPR in singleton pregnancies were 99.0% and 0.08%, respectively, for trisomy 21; 96.8% and 0.15% for trisomy 18; 92.1% and 0.20% for trisomy 13; 88.6% and 0.12% for monosomy X and 93.8% and 0.12% for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR was 94.4% and the FPR was 0% for trisomy 21. The authors concluded that screening for aneuploidies analyzing cfDNA in maternal blood provides effective screening for trisomies. The same group of authors updated the meta-analysis with data from 37 studies published since the initial review. The studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome. Weighted pooled DR and FPR in singleton pregnancies were 99.2% and 0.09%, respectively, for trisomy 21; 96.3% and 0.613% for trisomy 18; 91.0% and 0.13% for trisomy 13; 90.3% and 0.23% for monosomy X and 93.0% and 0.14% for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR for trisomy 21 was 93.7% and the FPR was 0.23% (Gil et al., 2015). In a 2017 update, weighted pooled DR and FPR in singleton pregnancies were 99.7% and 0.04%, respectively, for trisomy 21; 97.9% and 0.04% for trisomy 18; 99.0% and 0.04% for trisomy 13; 95.8% and 0.14% for monosomy X and 100% and 0.004% for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR for trisomy 21 was 100% and the FPR was 0.0%. This meta-analysis also highlighted low-risk and mixed-risk populations. The overall incidence of aneuploidy was higher than anticipated for the low or mixed risk population. When studies were broken out by technology, the sample sizes for the different types of tests were individually low. This suggests that more data is needed to determine the positive predictive value for the low risk or mixed risk populations (Gil et al., 2017).

Dar et al. (2014) reported clinical experience of a SNP-based NIPT in high- and low-risk women. Samples were received from 31,030 patients, 30,705 met study criteria and 28,739 passed quality-control metrics and received a report detailing aneuploidy risk. Samples that passed quality control were analyzed for trisomy 21, trisomy 18, trisomy 13, and monosomy X. Results were reported as high or low risk for fetal aneuploidy for each chromosome. Follow-up on outcome was sought for a subset of high-risk cases. False-negative results were reported voluntarily by providers. PPV was calculated from cases with an available prenatal or postnatal karyotype or clinical evaluation at birth. In all, 507 patients received a high-risk result for any of the four tested conditions (324 trisomy 21, 82 trisomy 18, 41 trisomy 13, 61 monosomy X; including one double aneuploidy case). Within the 17,885 cases included in follow-up analysis, 356 were high risk, and outcome information revealed 184 (51.7%) true positives, 38 (10.7%) false positives, 19 (5.3%) with ultrasound findings suggestive of aneuploidy, 36 (10.1%) spontaneous abortions without karyotype confirmation, 22 (6.2%) terminations without karyotype confirmation, and 57 (16.0%) lost to follow-up. This yielded an 82.9% PPV for all aneuploidies, and a 90.9% PPV for trisomy 21. The overall PPV for women aged ≥ 35 years was similar to the PPV for women aged < 35 years. Two patients were reported as false negatives. The authors reported that the results suggest the clinical performance of this SNP-based NIPT in a mixed high- and low-risk population is consistent with performance in validation studies. However, the authors also noted that the main limitation of the study is the incomplete follow-up data, particularly on low-risk patients. This lack of follow-up precludes precise calculation of sensitivity and specificity and determination of NPV.

In a multicenter comparative study, Bianchi et al. (2014) evaluated the performance of noninvasive prenatal testing using MPS of cfDNA in maternal blood for detecting fetal aneuploidy in low-risk women. The primary series included 1,914 women with an eligible sample, a singleton fetus without aneuploidy, results from cfDNA testing and a risk classification based on standard screening. For trisomies 21 and 18, the false positive rates with cfDNA testing were significantly lower than those with standard screening (0.3% versus. 3.6% for trisomy 21; and 0.2% versus. 0.6% for trisomy 18). The use of cfDNA testing detected all cases of aneuploidy (5 for trisomy 21, 2 for trisomy 18 and 1 for trisomy 13; NPV, 100%. The PPVs for cfDNA testing versus standard screening were 45.5% versus 4.2% for trisomy 21 and 40.0% versus 8.3% for trisomy 18. Further studies are needed to determine the clinical validity of sequence-based fetal aneuploidy testing in this patient population.

Using the same cohort of patients as noted in the 2011 publication below, Palomaki et al. (2012) reported additional data indicating that maternal plasma cfDNA sequencing also has the capability to detect other aneuploidies, such as trisomy 18 and trisomy 13. Sixty-two pregnancies with trisomy 18 and 12 with trisomy 13 were included in the analysis. Among the 99.1% of samples interpreted, observed trisomy 18 and 13 DR were 100% (59/59) and 91.7% (11/12) with FPR of 0.28% and 0.97%, respectively.

In the largest and most comprehensive study to date, Palomaki et al. (2011) evaluated the analytic validity of a noninvasive prenatal screening test for Down syndrome that measures circulating cfDNA in maternal plasma. Test results were compared to those obtained after chorionic villus sampling or amniocentesis. A total of 4,664 pregnant women, each considered at high risk for having a child with trisomy 21 (based on maternal age, screening test results and/or ultrasound results), were recruited from 27 prenatal diagnostic centers and included in this blinded, nested case-control study. Of the 4,664 cases, 279 (6%) were excluded from the analysis for various reasons. Of the remaining cases, 218 (5%) fetuses were diagnosed with trisomy 21 based on the results of invasive testing. The first 212 cases were selected for analysis. Fetal karyotyping was compared with an internally validated, laboratory-developed test based on next-generation sequencing. Down syndrome DR was 98.6% (209/212), the FPR was 0.20% (3/1471) and the testing failed in 13 pregnancies (0.8%). The authors concluded that, when applied to high-risk pregnancies, measuring maternal plasma DNA detects nearly all cases of Down syndrome at a very low FPR. This method can substantially reduce the need for invasive diagnostic procedures and attendant procedure-related fetal losses.

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 (Vanadis website). It is noted on the website that the Vanadis system is for research purposes only. 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.

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, yrisomy 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 standard deviations 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 detection rates. 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 1,208 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 tetrasomy 21/trisomy 7 and monosomy 21/trisomy 21. 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 three 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 aren't 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 1,200 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 3,500 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 fetal fraction 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 fetal fraction measurable. Fetal fraction 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.

Sex Chromosome Aneuploidies (SCA)

In an effort 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. Authors 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 Clinical Utility Evaluation, Hayes evaluated the clinical utility of cfDNA testing for fetal SCA in individuals with twin or singleton pregnancies. Conventional prenatal screening tests are not intended to identify SCAs, and these are generally detected incidentally in women undergoing diagnostic testing related to advanced maternal age or other high-risk screening for common aneuploidies. Recent meta-analyses (Soukkhaphone et al., 2021, Gil et al., 2017) have indicated that additional high-quality studies are needed to evaluate the clinical performance of cfDNA for SCA. There were no meta-analyses identified for performance of fetal SCA in twin pregnancy. 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 2022).

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) 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.

Other Aneuploidies

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

Hayes (2021c, updated 2022) published a Clinical Utility Evaluation addressing the use of cfDNA screening for fetal rare autosomal trisomies 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 one 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 3,306 enrolled cases, 753 were analyzed only for chromosomes 21, 13, and 18. All others, 2,553, were analyzed for all chromosomes and for segmental subchromosomal abnormalities. Results were reported in 2,527 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, del 80/dup 8q, 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 21, 18 or 13 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 fetal fraction 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 sex chromosome aneuploidies, 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 Variants and Microdeletions

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

A 2022 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 SNP-based cfDNA screening for detection of 22q11.2 deletion syndrome was published by Dar et al. in 2022. 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 six 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 five nested deletions), which yielded a prevalence of 1 in 1,524. 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 ≥ 1 Mb 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 > 1 Mb 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 ≥ 5 Mb. 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 ~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 a fetal fraction 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 > 6 Mb could be found, and few < 6 Mb unless maternally inherited. They concluded that because standard NIPT can only detect the larger chromosomal rearrangements and requires knowledge of fetal fraction, 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, fetal fraction 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 cell-free fetal DNA 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 cell-free fetal DNA 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.

Wapner et al. (2015) estimated the performance of a SNP-based NIPT for five microdeletion syndromes. After validation of the SNP-targeted assay, a cohort of 469 test samples was evaluated. DR were 97.8% for a 22q11.2 deletion (45/46) and 100% for Prader-Willi (15/15), Angelman (21/21), 1p36 deletion (1/1), and cri-du-chat syndromes (24/24). FPR were 0.76% for 22q11.2 deletion syndrome (3/397) and 0.24% for cri-du-chat syndrome (1/419). No false positives occurred for Prader-Willi (0/428), Angelman (0/442), or 1p36 deletion syndromes (0/422). Further validation studies are needed to determine the sensitivity and specificity of this screening test for microdeletion syndromes.

Zhao et al. (2015) reported on the development of a novel approach to identifying microdeletions in NIPT. The team used shallow whole genome sequencing followed by a rigorous statistical approach, applying a circular binary segmentation (CBS) method, to detect consistently increased or decreased regions in the normalized data. Additionally, the log odds ratio was calculated to determine the likelihood of a microdeletion event being true at the measured fetal fraction. The methodology was tested on 183 samples with known outcomes derived from amniocentesis, of which NIPT results were obtained on 178 samples. Of these, 16 were known to have microdeletions and 162 were normal. The average fetal fraction was 9.2%. The test identified 13 of the 16 affected samples. Of the three false negatives, one was anticipated to be a trisomy 8. Further analysis of this case found that the sample originated from a low-level mosaicism case and the sample was exempted from further testing. The second false negative did identify an abnormality that was reported to be a maternal, rather than fetal event. Both the mother and fetus had the same 4q34 deletion. The third false negative is likely due to a low fetal fraction of 4.8%. In the 162 samples predicted to be normal, five positive results were found. Only the amniocentesis results were available for concordance, no other fetal tissue or information, so to further assess these five potential false positives, deeper sequencing was performed on the samples. One sample was reclassified as normal, but the other four had the same positive result. The authors concluded that their algorithm correctly identified 17 of 18 cases with microdeletions and 156 of 157 unaffected cases, and the limitations of the approach include fetal fraction, microdeletion size, and the variability in the even region.

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.

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 fetal fraction 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 fetal fraction 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 cell free DNA 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 fetal fraction. 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

The proportion of fetal cell-free DNA 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. The lower limit of fetal cell-free DNA is 4%. Repeating an NIPT due to low fetal fraction is controversial, as before 20 weeks of gestation, the fetal fraction increases < 0.1% per week (Gregg et al., 2016). There is insufficient evidence to support the use of repeat NIPT screening due to low fetal fraction results. The limited studies available have not provided clinical utility to support this 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 fetal fraction (LFF) in prenatal cfDNA testing with adverse pregnancy outcomes. The authors note that LFF 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 LFF 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 6,375 pregnancies. All pregnancies had undergone cfDNA testing in the first or early second trimester. Regarding cutoff for LFF, two studies used 4%, two studies used the 5th and 25th percentiles and one study used a variety of varying cutoff values. Associations with LFF in prenatal cfDNA testing included hypertensive disease of pregnancy, small for gestational age newborns and preterm birth. Results for association of LFF and gestational diabetes mellitus were conflicting. The researchers concluded that LFF 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 LFF 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 LFF 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 fetal fraction 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 fetal fraction 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 fetal fraction (FF). Risks for trisomy 13, 18, and triploidy were evaluated using an FF-based algorithm. For each sample, an FF z-score (number of standard deviations 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 fetal fraction 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. A fetal fraction 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 1,148 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% (564/1,148) had a high score associated with a ≥ 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 fetal fraction is less than 2.8%, or the fetal fraction 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 fetal fraction 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 fetal fraction 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 fetal fraction 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 fetal fraction 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, standard deviation 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 1,000, and the low-risk group (LR) had a risk of trisomy 21 lower than 1 in 1,000. 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 (NT), 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 prospective cohort, systematic review and meta-analysis was performed by Yang et al. (2019) 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 cell-free DNA 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 cell-free DNA 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 cell-free DNA for RhD genotyping is an accurate and reliable tool for fetal immunoprophylaxis.

Saramago et al. (2018) conducted a health technology assessment of the use of cell-free DNA 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 at this time 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.

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 fetal fraction. 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 fetal fraction 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 fetal fraction 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 fetal cell-free DNA. Samples were collected from 49 couples at risk to have a child with beta-thalassemia, and genomic DNA was evaluated from the parents, cell-free fetal DNA 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 cell-free fetal DNA (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.

Byrou et al. (2018) presented their approach to developing a sensitive and reproducible assay for identifying paternally inherited beta-thalassemia genes in cell-free fetal DNA. Seventeen couples that were β-thalassemia carriers at a risk for having a child with β-globin gene disorder participated and were counseled on the 11th week of pregnancy about the study. Consent was obtained. Cell-free DNA was extracted from maternal plasma, and COLD PCR was initially used for amplification, which preferentially amplifies minor alleles that are present in mixtures with excess major alleles. A panel of 49 SNPs located on the beta-globin cluster on chromosome 11 and exhibiting high degree of heterozygosity (> 6%) in the Cypriot population was used for HBB mutation detection. COLD PCR did not provide satisfactory results, so the authors modified the protocol to utilize temperature gradient (TG) COLD PCR. Using TG COLD PCR allowed for the correct identification of the paternal HBB alleles in the cell-free fetal DNA in all cases, which was concordant with CVS analysis. The authors concluded that using TG COLD PCR can be a cost-effective approach for all labs and these results are an encouraging step forward in developing a NIPT assay for beta-thalassemia.

Duchenne (DMD) and Becker (BMD) muscular dystrophy are X-linked disorders caused by mutations in the dystrophin gene, and DMD is found in 1 in 3,500 male births. Most cases are caused by large deletions of the dystrophin gene (60-65%), small mutations (25-30%) and the rest by partial deletions (5-10%). Parks et al. (2016) reported on developing a test using cell-free fetal DNA to provide a screening tool for pregnant women. They developed an assay using massively parallel sequencing on an Illumina MiSeq by targeted capture enrichment of single nucleotide polymorphisms (SNPs) across the dystrophin gene, which then underwent relative haplotype dosage analysis. They recruited seven healthy pregnancies and two pregnant DMD carriers to the NIPSIGEN study ('NIPSIGEN: clinical translation of NIPD for SGDs'; REC approval number: 13/NW/0580). Tissue from the healthy donor CVS sample and blood from the DMD carriers was used to make a reference haplotype. When the fetal fraction was > 4%, the results from the NIPT test matched the fetal sample in all cases, including a dystrophin gene recombination event in one patient. The authors conclude that this initial validation study demonstrates that screening for DMD through NIPT can be accurate and reliable.

Twin Gestation

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 sex chromosome aneuploidies 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 and 18 and sex chromosome aneuploidies 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 21, 18 and 13 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, 10 had trisomy 18 and two had trisomy 13 identified by pre- or post-natal 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 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 6,290 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. Studies 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.

In a Hayes Clinical Utility Evaluation (2021d, updated 2022), the use of cfDNA screening for fetal trisomy 13, 18, and 21 in twin pregnancies was assessed. 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 rest for individuals with a twin pregnancy may reduce unnecessary invasive diagnostic procedures, overall the evidence base is limited in quantity and quality.

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 six 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 fetal fraction was 12.2% (range of 3%-36%) and all nine samples with only 3% fetal fraction 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%, 010%, 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.

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 cell-free DNA. This was the largest to date cfDNA multi-gestation 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, 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-. Fetal fraction 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 3 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.

Twin Zygosity

The evidence is insufficient to support the use of NIPT in twin zygosity due to the lack of prospective studies that establish clinical utility. As testing is advanced it may be possible to use NIPT in the future.

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 single nucleotide polymorphism (SNP) based NIPT to determine zygosity (monozygotic MZ vs. dizygotic DZ) 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. Monochorionicity (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 for twin-twin transfusion syndrome (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. Prospective studies were recommended by the authors to establish the clinical utility of SNP-based NIPT in twins with assignment of both zygosity and aneuploidy risk.

Qu et al. (2013) studied the ability of MPSS to assess twin zygosity using cffDNA. Eight women with twin pregnancies were consented for the study. The sets included four dichorionic, diamniotic twins, three monochorionic diamniotic twins, and one monochorionic monoamniotic. Cord blood was collected separately from each twin at the time of delivery. Microarray genotyping was used to assess the cffDNA, and regions of DNA heterozygosity were compared within each sample. Little regional variation was expected for identical twins, while greater variation was expected for fraternal twins. This method was able to accurately identify the monozygotic and dizygotic twin pregnancies, and in dizygotic twins could determine the contribution of each twin to the fetal fraction.

Leung et al. (2013) utilized MPSS of eight twin pregnancies, six of which had euploid twins, and two of which were known to have one aneuploid twin, and eleven known euploid singleton pregnancies. Their goal was to assess the ability of cffDNA to provide an individualized assessment of trisomies in twins, as well as zygosity and fetal fraction. Here we report on the zygosity results. Zygosity was determined using an algorithm to analyze the ratio of fetal-specific allele to major allele for specific loci. For identical twins it is expected that the fetal fraction calculated would be the same across all loci, but for fraternal twins, the fetal fraction would vary across various loci. Using this information, the algorithm correctly identified four twin pregnancies as identical, and the other two twin pregnancies as fraternal. The authors concluded that knowing the zygosity not only contributes to better aneuploidy prediction in twin gestations for NIPT testing, but also has additional clinical utility since monozygotic pregnancies have a higher complication rate and knowing this information early could help the clinician better monitor the pregnancy. In addition, the authors identified the need for more studies before this could be used clinically.

Clinical Practice Guidelines

American College of Medical Genetics and Genomics (ACMG)

An ACMG position statement addresses the advantages and disadvantages of noninvasive prenatal screening for fetal aneuploidy and offers recommendations for pre- and post-test genetic counseling. The statement includes the following recommendations:

- Provide up-to-date, balanced, and accurate information early in gestation to optimize patient decision making, independent of the screening approach used.
- Laboratories should work with public health officials, policymakers, and private payers to make NIPT, including the pre- and post-test education and counseling, accessible to all pregnant women.
- Allow patients to select diagnostic or screening approaches for the detection of fetal aneuploidy and/or genomic changes that are consistent with their personal goals and preferences.
- Inform all pregnant women that diagnostic testing (CVS or amniocentesis) is an option for the detection of chromosome abnormalities and clinically significant copy-number variants.
- Inform all pregnant women that NIPT is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes).
- Offer diagnostic testing for a no-call result due to low fetal fraction. Repeat testing is not appropriate.
- Offer aneuploidy screening other than NIPT in women with extreme obesity.
- Labs should always report fetal fraction results and report the reason for a no-call result.
- Do not offer NIPT for autosomal aneuploidies other than chromosomes 13, 18, and 21.
- Do not offer NIPT to screen for genome wide copy number variants.
- Offer aneuploidy screening other than NIPT for patients with a history of bone marrow or organ transplantation from a male donor or donor of uncertain biological sex.

ACMG emphasizes that all genetic screening has residual risk and there are pros and cons to any screening approach (Gregg et al, 2016).

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 (NT), ultrasound or cell-free DNA 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.
- Cell-free DNA 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, cell-free DNA 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 cell-free DNA 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.
- Cell-free DNA 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 cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. As such, it is difficult to determine an accurate detection rate 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 cell-free DNA is used. In these cases, individuals should be counseled and diagnostic testing should be offered.
- When unusual or multiple aneuploidies are detected by cell-free DNA, affected individuals should be referred for genetic counseling and maternal–fetal medicine consultation. (ACOG, 2023).

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

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 multi fetal 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. Cell-free DNA 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 regarding cfDNA include:

- Prenatal genetic screening (serum screening with or without nuchal translucency (NT) ultrasound or cell-free DNA 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.
- Cell-free DNA 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, cell-free DNA 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 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.

Level C recommendations 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 cell-free DNA is used.
- Patients with unusual or multiple aneuploidies detected by cell-free DNA 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.

An ACOG practice advisory recognizes the emerging technology and availability of cell-free DNA 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 2021). For this reason, ACOG does not recommend single gene cfDNA screening in pregnancy.

In Committee Opinion 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)

In a 2021 Position Statement, the ISPD (Palomaki et al.) addresses the use of cfDNA screening for Down syndrome in multiple gestation pregnancies. 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.

ISPD guidance on the use of cfDNA for aneuploidy screening noted that cfDNA should be a primary screening test offered to all pregnant women. The guidance also noted that it was appropriate as a secondary test for women found to be at high risk due to an abnormal serum screening result or ultrasound finding. cfDNA is significantly better than conventional screening with a much high odds of being affected given a positive result (OAPR). The authors concluded that while the performance of the test has been initially established in high-risk women, there was now sufficient evidence to support the use of testing in women of average risk (Benn et al., 2015).

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.

An NSGC practice guideline (Wilson et al., 2013) provides a summary of screening and diagnostic testing options for chromosome aneuploidy. A decision tree and comparison tables are included to help providers select the test which best suits their patient's needs. The guideline also states that referral to a genetic counselor or other qualified provider may be appropriate if a patient could benefit from additional discussion prior to making a decision regarding screening and diagnostic testing options. NIPT is only recommended for patients from high-risk populations, including advanced maternal age, positive

screening test, abnormal ultrasound suggestive of aneuploidy or prior pregnancy with chromosome aneuploidy. It is recommended that a positive NIPT be followed by confirmatory diagnostic testing prior to making pregnancy decisions.

Society for Maternal-Fetal Medicine (SMFM)

In 2015 statement, SMFM recommends the following points of consideration regarding cfDNA testing for aneuploidy:

- cfDNA screening appears to be the most accurate test for trisomy 21.
- cfDNA screening does not screen for all chromosome conditions and will not identify risk for the range of disorders potentially identified with traditional screening.
- cfDNA screening has false positive and false negative results which is particularly true for lower risk women.
- Diagnostic confirmation with CVS or amniocentesis is recommended for women with abnormal cfDNA results. Irreversible decisions for pregnancy termination should not be undertaken based on cfDNA results.
- A negative cfDNA result does not definitively rule out trisomy 21 or other chromosome abnormalities.
- Women with failed cfDNA have an increased risk for aneuploidy, need genetic counseling and should be offered diagnostic testing.
- SMFM recommends payers provide adequate reimbursement for genetic counseling services.
- All genetic screening is elective. Aneuploidy screening, diagnostic testing or no testing is a personal decision and any of these is a reasonable option. (SMFM 2015a).

In a statement clarifying recommendations regarding cfDNA testing, SMFM asserted that it does not recommend that cfDNA aneuploidy screening be offered to all pregnant women. Limited data on the effectiveness and clinical utility for improving patient outcomes preclude a recommendation that cfDNA be actively offered to all pregnant women. However, SMFM believes that the option should be available to women who request additional testing beyond what is currently recommended by professional societies (SMFM, 2015b).

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 March 22, 2023)

Additional Product Information

- Harmony™ Prenatal Test (Ariosa® Diagnostics/Roche)
- MaterniT21® PLUS (LabCorp®/Sequenom Laboratories)
- Panorama™ Prenatal Test (Natera™ Inc.)
- QNatal® Advanced (Quest Diagnostics™)
- SensiGene (LabCorp®/Sequenom Laboratories)
- Vanadis™ NIPT Test (Vanadis Diagnostics)
- Verifi® Prenatal Test (Illumina®, Inc.)
- VisibiliT™ (LabCorp®/Sequenom Laboratories)
- Vistara™ (Natera™ Inc.)

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Policy History/Revision Information

Date	Summary of Changes
04/01/2024	**Applicable Codes** • Updated list of applicable CPT codes to reflect quarterly edits; added 0449U **Supporting Information** • Archived previous policy version CS085.Z

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.

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