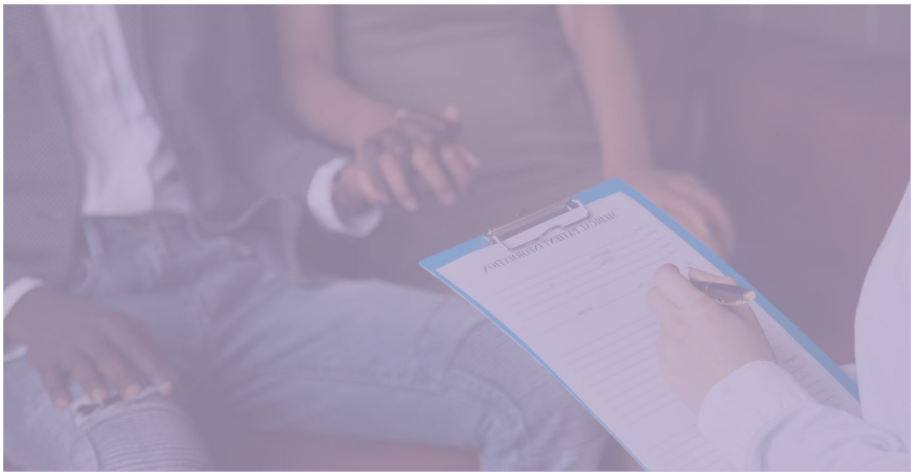
Prenatal Screening Provider Handbook

California Prenatal Screening Program

What Providers Should Know



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Prenatal Screening Provider Handbook

Table of Contents

Table of Contents (continued)

The California Prenatal Screening Program

INTRODUCTION

The California Prenatal Screening (PNS) Program is a comprehensive public health service that makes prenatal screening available to all pregnant individuals in the state who want it. Each year approximately 340,000 pregnant individuals choose prenatal screening through the PNS Program. The PNS Program is administered by the Genetic Disease Screening Program (GDSP) of the California Department of Public Health (CDPH).

Pregnant individuals decide if they want to participate in the PNS Program. If they do participate, Medi-Cal or private health insurance must cover all program fees, with only a few exceptions (for self-insured employers or out-of-state health plans). In addition to screening, for patients with positive screening results, the program fee includes high-quality follow-up services, such as genetic counseling, ultrasound, and diagnostic tests at state-approved Prenatal Diagnosis Centers (PDCs).

The PNS Program also provides participant and provider educational materials focused on the benefits and limitations of screening. A program e-newsletter with important updates is published on an as-needed basis. The PNS Program additionally maintains contracts and provides quality control monitoring for all state-approved PDCs.

It is important that providers understand the PNS Program and are able to explain it easily to pregnant individuals. This Prenatal Screening Provider Handbook is a tool to help providers understand the PNS Program.

California Prenatal Screening Program benefits

- **The online CalGenetic Portal** (https://calgenetic.cdph.ca.gov/) that allows expedited screening test orders and access to screening results;
- **Patient and provider education booklets** at no cost, including the Prenatal Screening Patient Booklet, Provider Handbook, screen-positive and
- **Patient and provider education videos** at no cost;
- **Patient consent and refusal documents** at no cost;
- **PNS Program label stationery** to use when printing the cfDNA "Consent and Electronic Order Confirmation" and the Maternal Serum Alpha-Fetoprotein (MSAFP) screening test "Consent and Electronic Order Confirmation" from the CalGenetic Portal;
- **Supplies to draw and mail MSAFP serum samples** at no cost; and
- **Follow-up services at state-approved PDCs.** The PDCs are independent perinatal clinics that are authorized to see referred, screen-positive patients. See current list of state-approved PDCs on the **PNS Program Information for Providers web page** (https://bit.ly/PNS4Providers).

Summary: Prenatal screening tests offered

The PNS Program offers two screening blood tests to pregnant individuals with either singleton or twin gestations, to identify those who are at increased risk for carrying a fetus with certain fetal chromosomal and structural anomalies. These blood tests are drawn in the first and second trimester. Because screening does not diagnose fetal anomalies, but only estimates the risk for them, diagnostic testing is needed to confirm fetal anomalies.

The PNS Program offers two screening blood tests to pregnant individuals to identify those who are at increased risk for carrying a fetus with certain fetal chromosomal and structural anomalies.

Cell-free DNA (cfDNA) screening can be ordered on or after 10 weeks 0 days through term. The PNS Program recommends screening from 10 weeks 0 days through 21 weeks 0 days since some follow-up diagnostic services may not be available after 24 weeks 0 days.
However, participants will be able to get cfDNA screening through the end of the pregnancy.

cfDNA screening identifies pregnancies at risk for the following chromosomal anomalies:

- Trisomy 21 (Down syndrome)
- Trisomy 18 (Edwards syndrome)
- Trisomy 13 (Patau syndrome)
  - Turner syndrome (45, X)
  - Klinefelter syndrome (47, XXY)
  - Trisomy X (47, XXX)
  - XYY (47, XYY)

Maternal serum alpha-fetoprotein (MSAFP) screening is completed from 15 weeks 0 days to 21 weeks 0 days. MSAFP screening identifies the risk of the following type of structural anomalies:

- Open neural tube defects

Summary: Prenatal screening providers role and responsibilities

Prenatal screening providers play an important role in prenatal screening for fetal chromosomal and structural anomalies. California law[1] requires providers to offer pregnant individuals prenatal screening to identify those who are at increased risk for carrying a fetus with one of the fetal chromosomal and structural anomalies screened by the PNS Program.

Providers are also legally required to provide the Prenatal Screening Patient Booklet to every pregnant individual to whom they provide care. Whether a pregnant individual decides to participate in the PNS Program or not, providers should get a signed form confirming their choice. Provider responsibilities within the PNS Program include the following:

1. Deliver patient education. It is the provider's responsibility to ensure that pregnant individuals understand the basics of prenatal screening when it is offered, as well as **to provide the Prenatal Screening Patient Booklet at the first prenatal care visit**. Providers should use plain language and answer all patient questions when discussing it.

It is especially important that patients understand the following:

- Prenatal screening through the PNS Program is voluntary;
- The PNS Program only screens for eight types of chromosomal and structural anomalies;
- No screening is 100% accurate;
- Prenatal screening is different from diagnostic testing; and
- Follow-up services are offered if a patient receives screen-positive screening test results from the PNS Program.

Sex chromosome aneuploidies (SCAs)

As of April 2024, the California Prenatal Screening (PNS) Program has added to its screening panel the most common SCAs, also known as X and Y chromosome variations. In this document, the term SCA will be used henceforth. SCAs are genetic conditions that involve an atypical number of sex chromosomes. Aneuploidies can occur due to errors during meiosis, resulting in extra or missing sex chromosomes.

Adding SCAs to the California PNS panel is in keeping with the American College of Medical Genetics and Genomics (ACMG) publication from December 2022 that "strongly recommends" offering noninvasive prenatal screening for SCAs, in addition to trisomy 21, 18, and 13.

Educating patients on SCAs

- Terminology

The California PNS Program uses the term “X and Y chromosome variations” in pregnant individual health education. Disability rights advocates have found the term “sex chromosome aneuploidy” to be confusing and stigmatizing. “X and/or Y chromosome variations” is technically more accurate but cumbersome. Community members, providers, and disability rights advocates collaborated in workshops with PNS Program staff to enhance understanding on terminology used by people affected by SCAs.

- Prescreening education

When explaining SCAs, with pregnant individuals, one key aspect to emphasize is the wide range of presentations associated with SCAs, which can vary from mild to severe cases. By highlighting this variability, you can help pregnant individuals understand that the effects of SCAs may differ significantly among individuals. In addition to SCAs variability, the topics of Fetal Sex and Follow Up Diagnostic Services should be discussed with pregnant individuals that are voluntarily choosing to participate in the PNS program.

Providers are obligated to tell pregnant individuals that SCA screening may not be as accurate when compared to the other genetic conditions and birth defects the PNS Program screens for (trisomy 21, 18, 13, and neural tube defects).

It is your responsibility to share information about the California Newborn Screening (NBS) Program with your pregnant patients as per the California Code of Regulations (CCR, Title 17, Section 6504). Please order, or download and print, the **Important Information for Parents trifold** (http://www.cdph.ca.gov/NBSIIP) to distribute to each patient.

Note that the Prenatal Screening Patient Booklet also includes information about the NBS Program. This is to give pregnant individuals advance notice that their newborn will receive screening for over 80 serious genetic conditions shortly after birth. The NBS Program Important Information for Parents trifold is available in English, Spanish, Chinese and other languages.

2. Offer screening. Providers are obligated to offer the PNS Program screening tests to all pregnant patients. They must discuss the types of screening tests available and appropriate with the patient, considering their unique medical, pregnancy, and family histories.

[1] California Health and Safety Code (Division 106, Part 5, Chapter 1: Article 4, Sections 125050-125070; Article 1, Sections 124975-124980)

3. Get patient consent or refusal decline to participate. The provider must have the patient provide a signature on the printed PNS order confirmation after each order is placed on the CalGenetic Portal**. If a blood sample is not accompanied by the participants signed consent, the sample will not be tested.** If the patient declines participation, they should sign a decline form, found on the PNS Program Information for Providers Page (http://cdph.ca.gov/pns), as well as the online appendices of this handbook. The PNS program will maintain a copy of the patient signature of consent to screening. For patients that decline cfDNA or MSAFP screening, a copy of the signed decline form should be placed in the patient's medical record.

4. Offer Sexual Orientation and Gender Identity (SOGI) information survey. California Health and Safety Code Title 2, Division 1, Chapter 5, section 8310.8 – (a) (1,) requires various state agencies that provide health and human services to members of the LGBT community to collect voluntarily provided information about Sexual Orientation and Gender Identity (SOGI) in the regular course of collecting other types of demographic data. The Prenatal Screening Patient Booklet contains a URL and QR code on Page 11 that links to the PNS Program's Sexual Orientation and Gender Identity (SOGI) information survey (https://forms.office.com/g/LRUWGVE7Xx).

The provider should specifically mention the links and indicate that the confidential SOGI information survey is available for the patient to provide unidentifiable information for data collection purposes. Providers should stress that patient submission of the survey is helpful but voluntary and will not affect any prenatal risk assessment or results interpretation.

5. Order screening tests. Providers must order screening tests using the CalGenetic Portal (https://calgenetic.cdph.ca.gov).

6. Order supplies. Providers must order and distribute to patients the free patient education booklets. They are also responsible for ordering in advance MSAFP and cfDNA screening blood collection kits if blood is drawn in their office. Providers can order supplies and MSAFP blood kits on the PNS Program Supplies Ordering web page (https://bit.ly/PNSSupplies).

7. Collect blood samples. **cfDNA:** If providers prefer to send their patients to an outside draw station, detailed instructions for each respective cfDNA screening laboratory is available on the PNS Program website at: PNS Program Information for Providers web page (https://bit.ly/PNS4Providers), or the CalGenetic Portal Resource web page (http://calgenetic.cdph.ca.gov).

MSAFP: Providers may send their patient to any draw station in California that collects blood for the PNS Program. If providers prefer to send their patients to an outside draw station, detailed instructions for MSAFP specimen collection is available on the: PNS Program Information for Providers web page (https://bit.ly/PNS4Providers), or the CalGenetic Portal resource web page.

8. Notify patients of screening results.
Providers must notify patients about screening test results, whether positive or negative. For screen-positive results, the PNS case coordinator will help the provider refer a patient to a state-approved PDC if that is the patient's decision.

Prenatal Screening Case Coordinators

PNS case coordinators support prenatal screening providers with program implementation and case management. PNS case coordinator offices are located throughout California. Each prenatal screening provider is assigned a PNS case coordinator by zip code. The prenatal screening provider should rely on the PNS case coordinator as the primary source of information regarding the PNS Program. The PNS case coordinator's phone number is included on every result mailer. A PNS case coordinator will contact a provider office when there is missing or incomplete information on a screening test order or to verify information for certain results such as positives, samples drawn too early or too late, or any non-negative screening test result.

PRENATAL SCREENING TESTS

In 2022, the PNS Program simplified its screening test choices to include cfDNA screening and MSAFP screening tests. It is strongly recommended that providers encourage eligible patients who opt for prenatal screening to get both screening tests since each screens for different fetal chromosomal and structural anomalies.

Cell-free DNA (cfDNA) screening test

The cfDNA screening test, also known as a noninvasive prenatal screening test (NIPT), is used to screen for certain chromosomal anomalies in a fetus: trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome); and trisomy 13 (Patau syndrome), Turner syndrome (X), Klinefelter syndrome (XXY), trisomy X (XXX) and XYY.

Benefits of using cfDNA screening include the following:

- cfDNA provides higher sensitivity and lower false-positive rates than the previous biochemical methodology, resulting in fewer screen-positive cases unnecessarily referred for follow-up diagnostic services;
- The gestational age window will allow screening as early as 10 weeks of gestation;
- cfDNA can report results for trisomy 13, Turner syndrome (X), Klinefelter syndrome (XXY), trisomy X (XXX) and XYY as opposed to biochemical screening.

Prenatal cfDNA screening detects small fragments of fetal DNA released by placental cells into the blood stream of a pregnant individual. cfDNA screening looks for a relative increase or decrease in specific regions of the fetal DNA that would suggest the presence of a chromosome anomaly.

While the sensitivity and specificity of cfDNA screening is high, it is not 100% accurate. False-positive and false-negative cfDNA screening results have been reported in peer reviewed articles.

Results can assess the fetus's increased or decreased risk for a chromosomal anomaly. If the results suggest an increased risk, additional diagnostic testing is needed.

cfDNA analysis explained

Both false negatives and false positives occur with cfDNA testing. False negatives can occur because of low levels of fetal DNA in the maternal serum related to early fetal age, maternal obesity, or because of a failure to collect a sufficient volume of cfDNA. Some reasons for false positive results include maternal malignancies, maternal mosaicism for aneuploidy, or placental mosaicism.

Fetal fraction

The fetal fraction is the percentage of total cfDNA in a sample derived from the fetus. The fetal fraction can affect the ability of cfDNA to screen for fetal aneuploidy. The majority of cfDNA in maternal blood originates from the pregnant individual; however, about 10 to 20% is composed of fetal DNA originating from the placenta and freely circulating in maternal plasma.

Prenatal cfDNA screening has been shown to be less effective if the pregnant individual has the following attributes:

- Is pregnant with multiples
- Has a body mass index of 30 or higher (obesity)
- Is pregnant via in vitro fertilization (IVF), (either the pregnant individual's egg or a donor egg)
- Is pregnant as a gestational carrier
- Is less than 10 weeks pregnant
- Is taking certain blood thinners

About 1 to 5% of prenatal cfDNA screening tests do not yield any result, possibly due to the sample not having enough of the fetal DNA or other material necessary for the test.

Low fetal fraction could also be indicative of an aneuploidy.

There are no guidelines from scientific societies recommending an optimal gestational age for cfDNA sampling in cases with obesity.

cfDNA does not replace the need for invasive diagnostic testing (e.g., chorionic villus sampling [CVS] or amniocentesis) in high-risk pregnancies with a higher risk of aneuploidy.

Screening for fetal sex

The PNS Program includes screening for fetal sex on all samples. Participants decide if they want to know the fetal sex, and this choice is indicated when ordering the cfDNA screening test. Fetal sex is predicted based on chromosomes detected in the blood sample. The cfDNA screening is not 100% predictive of fetal sex. Discrepancies do arise, and there could be technical reasons why sex is reported incorrectly.

The cfDNA screening is not 100% predictive of fetal sex.

These reasons could include sample contamination, sex chromosome aneuploidy, and low fetal fraction. Discrepancies could lead to discordant results between ultrasound results and the cfDNA fetal sex results. A fetal sex indeterminant result can happen when there is not enough fetal DNA to examine.

Sex chromosome abnormalities

The PNS Program, currently, does not include screening for sex chromosome anomalies or microdeletions. Providers can order these screens, if they choose, as an additional screening that can be ordered through a private laboratory. Information about which state-contracted cfDNA laboratories offer additional testing and how to obtain additional tests is available on the CalGenetics Portal resource web page and on the **PNS Program Information for Providers web page** (https://bit.ly/PNS4Providers). Additional screens will require separate billing to the patient or their third-party payor, and any necessary follow-up services will not be paid for by the PNS Program.

Maternal serum alpha-fetoprotein (MSAFP) screening test

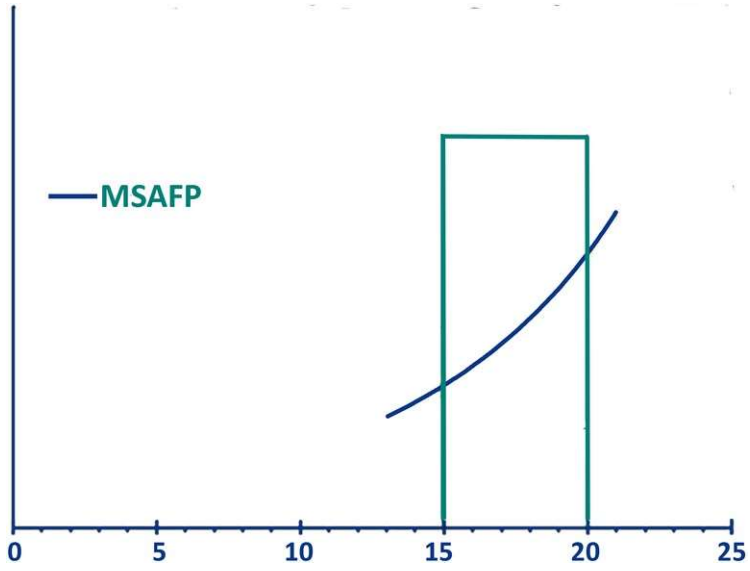
AFP is a protein produced mainly in the fetal liver and released into the maternal serum (MSAFP) and amniotic fluid. A small amount crosses the placenta and becomes measurable in the maternal serum towards the end of the first trimester. Levels rise steadily through the second trimester. In most fetuses affected with open spina bifida or anencephaly, an increased amount of MSAFP enters the amniotic fluid. It subsequently causes a higher-than-expected level of MSAFP in the maternal serum.

Multiple of the Median (MoM) for MSAFP

The PNS Program has established medians for MSAFP for each day from 15 weeks 0 days through 21 weeks 0 days gestation. For each blood sample received, the analytic value for the serum marker tested is converted to a multiple of the median (MoM) based on the gestational age at blood collection. The median level for each day equals a MoM of 1.00. For example, an MSAFP result of 1.50 MoM means the patient has one and a half times the median level of MSAFP.

A MoM may be adjusted for patient race/ethnicity, weight, and diabetic status to give a more accurate risk assessment.

Chart 1: Concentration of serum MSAFP marker varies by gestational age



AFP is a protein produced mainly in the fetal liver and released into the maternal serum (MSAFP) and amniotic fluid. Levels rise steadily through the second trimester.

Increased risk for an open neural tube defect

The PNS Program uses only the second trimester MSAFP analyte for an assessment of increased risk for an open neural tube defect (NTD). A patient is classified as screen positive for a fetus with an open NTD when the MSAFP value is elevated over the selected cutoff. This is currently > 2.50 MoM for a pregnancy with a single fetus, or > 4.50 MoM for a pregnancy with two fetuses (see Appendix B for more information on NTDs).

Screen-positive rate with MSAFP

Among program participants, 1% are initially screen positive for NTD. Among individuals who have prenatal screening and diagnostic services, the PNS Program identifies approximately 97% of fetuses with anencephaly, 80% with open spina bifida, and 85% of abdominal wall defects (AWDs, gastroschisis and omphalocele). Other reasons for this screen-positive result are underestimation of gestational age, multiple gestation, and fetal demise.

Some apparently normal pregnancies have MSAFP levels over the selected cutoff of 2.50 MoM. Elevation of MSAFP is frequently associated with a high-risk pregnancy even if no NTD is found. An increased risk for low birth weight, preterm delivery, and fetal demise is associated with otherwise unexplained high mid-trimester MSAFP values. Early identification of these high-risk pregnancies may facilitate better obstetrical management. The PNS Program does not cover costs associated with obstetrical management, additional testing, or treatment beyond prenatal diagnosis.

USING THE ONLINE CALGENETIC PORTAL

The online CalGenetic Portal (https://calgenetic.cdph.ca.gov/) is a web-based portal available to prenatal screening providers to place screening test orders and access real-time patient results for the PNS Program.

Providers should use the CalGenetic Portal to register, log in, or set up delegates to an account. In the CalGenetic Portal, providers will be able to:

- Save draft orders
- Submit orders
- Return to their orders inventory list
- View test status
- View prenatal screening results and messages from the PNS Program
- View any follow-up actions authorized
- Submit a "Confidential Report of an Outcome of Pregnancy"

ORDERING PRENATAL SCREENING TESTS

Consent and Electronic Order Confirmations

Providers will fill out the details of the screening test ordered using the CalGenetic Portal. They then print out the "Consent and Electronic Order Confirmation," which includes a line for a written patient signature and date. This is in place of the previously used Test Request Form (TRF).

Back-up, fillable PDFs, known as "Consent and Order Forms," are posted on the PNS Program Information for Providers web page (https://bit.ly/PNS4Providers). The provider may plan ahead for the eventuality of a power outage or loss of internet access and download and print several "Consent and Order Forms" to keep on hand. When needed, the patient can sign and date the paper "Consent and Order Form" instead of the "Consent and Electronic Order Confirmation."

The original, signed copy of the "Consent and Order Form" must accompany the patient's blood sample when shipped to the analysis laboratory, and a copy should be placed in the patient's health record. The analysis laboratory performs the test and sends the lab results electronically to the PNS Program.

Providers who pre-print the "Consent and Order Form" PDF will be unable to view patient results on the CalGenetic Portal. A PNS form number is needed to access results electronically, which is only obtained when placing an order online. PNS case coordinators will not share PNS form numbers with providers or fax result copies directly to provider offices. Paper mailers of results will continue to be sent to the ordering provider's office.

If the cfDNA screening order is placed through the CalGenetic Portal, the provider will be able to independently display fetal sex screening results without further assistance from the PNS case coordinator. If the cfDNA screening order is placed using a pre-printed "Consent and Order Form," the provider must notify the PNS case coordinator for assistance with fetal sex screening results. There is no additional charge for revealing fetal sex after the specimen is processed.

Note that ordering screening tests involves one of two types of documents:

- Confirmations generated from the online CalGenetic Portal:
  cfDNA Consent and Electronic Order Confirmation MSAFP Consent and Electronic Order Confirmation
- Back-up, fillable, PDF consent and order forms to be downloaded and printed from the PNS Program website:
  cfDNA Consent and Order Form MSAFP Consent and Order Form

Placing Screening Test Orders

Providers must place orders online through the CalGenetic Portal in most cases. The provider or their delegates must print the patient's "Consent and Electronic Order Confirmation" from the CalGenetic Portal for the patient signature.

The PNS Program label stationery, used for printing the "Consent and Electronic Order Confirmation," includes four barcode labels. One will remain on the confirmation form, one will be placed on the photocopy of the insurance card, and one is placed vertically on the requested vial(s) of blood. (Note: one of the cfDNA laboratories currently requires two vials of blood to be drawn.) For details see the PNS Program cfDNA blood collection and order instructions for each respective laboratory on the **PNS Program Information for Providers web page** (https://bit.ly/PNS4Providers). The free PNS Program **PNS Program label stationery is available to order online** (https://bit.ly/PNSSupplies). Order requirements and clinician instructions for the cfDNA screening is slightly different than for the MSAFP screening. See the requirements and instructions for both below.

cfDNA order requirements and clinician instructions

- Review the Prenatal Screening Patient Booklet with the patient.
- If the patient wants prenatal screening, complete the screening order in the CalGenetic Portal. Use the gestational age calculator **(https://calgenetic.cdph.ca.gov/content/gdsp/en/portal-landing-page.html)** for the estimated due date.
- If the patient wants to know the fetal sex, the provider will need to indicate on the order that the fetal sex should be displayed in the results.
- Print the cfDNA "Consent and Electronic Order Confirmation" on to the PNS Program label stationery.

- The provider must confirm the patient (or a patient's representative) signs and dates the cfDNA "Consent and Electronic Order Confirmation."
- Make a copy of the patient's insurance card. Apply one barcode label from the cfDNA "Consent and Electronic Order Confirmation" to the copy of the insurance card.
- Assemble a packet for the patient, including the following:
    - cfDNA Order Patient & Blood Draw Instructions
    - cfDNA "Consent and Electronic Order Confirmation," signed and dated by the patient
    - Copy of insurance card (with barcode label attached)
- Instruct the patient to bring the above packet when they get their blood drawn.

Unless they are collecting blood samples directly in their office using a pre-ordered blood collection kit, providers should give information on the closest blood collection facility to the patient for the requested PNS screen (each cfDNA laboratory has their own specific instructions about how to locate an appropriate blood collection draw location). The signed patient consent form must accompany the blood sample to the analysis lab for processing.

MSAFP order requirements and clinician instructions

- Review the Prenatal Screening Patient Booklet with the patient
- If the patient wants prenatal screening, complete the screening order in the CalGenetic Portal. Use the **gestational age calculator** (calgenetic.cdph.ca.gov/resources) for the estimated due date.
- A patient may decline to have their MSAFP blood sample used for research by signing and checking the box on the MSAFP 'Consent and Electronic Order Confirmation" marked, "I decline to use my sample for research."
- Print the MSAFP "Consent and Electronic Order Confirmation" on to the PNS Program label stationery.
- The provider must confirm that the patient signs and dates the MSAFP "Consent and Electronic Order Confirmation."
- Make a copy of the patient's insurance card. Apply the barcode label from the MSAFP "Consent and Electronic Order Confirmation" to the copy of the insurance card.
- Assemble packet for the patient, which includes:
    - MSAFP Order Patient & Blood Draw Instructions
    - MSAFP "Consent and Electronic Order Confirmation" signed and dated by the patient
    - Copy of insurance card (with barcode label attached)
    - USPS prepaid MSAFP mailing label

Note: A USPS prepaid MSAFP mailing label can only be generated through the online CalGenetic Portal order submission page. The USPS prepaid MSAFP mailing label generated is dependent on the provider's FACILITY zip code used to place the order.

- Instruct the patient to bring the above packet when they get their blood drawn. Unless they are collecting blood samples, providers should give information on the closest blood collection facility to the patient. The original, signed patient consent form must accompany the blood sample to the analysis lab for processing.

Filling out the screening test order for an accurate result

Providers must fully and accurately complete the screening test order, filling out all fields. Erroneous or missing information may lead to an incorrect screening result. Please note the following details.

Date of birth: A patient's biological age is used to determine an individualized risk for carrying a fetus with Down syndrome, trisomy 18, trisomy 13, and Turner syndrome, Klinefelter syndrome, trisomy X and XYY.

Gestational age: The median level of MSAFP changes each day during pregnancy. It is important to choose the most accurate gestational dating method available. Providers should use only one method of gestational dating. Dating by Nuchal Translucency (NT) ultrasound is the most accurate. If an NT ultrasound was not performed, ultrasound dating by Crown Rump Length (CRL), Last Menstrual Period (LMP), or physical exam may be used. NT CRL is the most accurate dating method, followed by ultrasound dating based on biparietal diameter (BPD).

Weight: Heavier pregnant individuals have lower median values for MSAFP, while lighter pregnant individuals have higher median values.

Race: Some races have different median values. For example, Black pregnant individuals have higher medians for MSAFP. For cfDNA and MSAFP screening, the PNS Program will allow up to four races/ethnicities placed on the CalGenetic Portal when filling out the screening test "Consent and Electronic Order Confirmation." Providers should use the dominant races/ethnicities as self-identified by the pregnant individual. The PNS Program recommends including only the races that best represent 50% or more of the patient's genetic background. The PNS Program allows up to four races/ethnicities for those individuals whose genetic background is less than 50% of any one race or ethnicity, however.

Number of fetuses: cfDNA and MSAFP screening cannot be used in a pregnancy with more than two fetuses. There is not enough verifiable data on the accuracy of cfDNA and multiple gestations of more than two fetuses. The level of MSAFP is usually increased with a multiple gestation, and levels are approximately double for twins. There are no established MSAFP median values for more than two fetuses.

Diabetic status: The amount of MSAFP is usually lower in patients diagnosed with diabetes prior to and during pregnancy. "Diabetes alone does not seem to alter levels of fetal fraction or results of cfDNA; however, increasing rates of type 2 diabetes are found in women with obesity." [2]

Ovum donor:

- The ovum donor age must be provided for a separate MSAFP risk assessment of the ovum donor to be calculated. It is important to provide the age of egg(s) at the time of donation, even when the patient is using their own egg(s) at the time of collection, to obtain a separate risk assessment for MSAFP.

[2] Hopkins MK, Dugoff L. Screening for Aneuploidy in the Patient with Diabesity: Pearls and Pitfalls. Clinical Obstetrics and Gynecology. 2021 Mar 1;64(1):136-143.

- Whether the pregnancy has an ovum donor or uses their own eggs, the age of the egg(s) is required information for cfDNA analysis.

Fetal sex: Sex of the fetus will not be displayed within the results to the provider as a default. If the pregnant individual wants to know the fetal sex, then the provider will need to indicate on the order that the fetal sex should be displayed in the cfDNA screening results. If the pregnant individual changes their decision to know the fetal sex after the cfDNA order has been placed the provider can change the fetal sex check box from "no" to "yes" in the online order and instantly generate a new result. The provider must contact their PNS case coordinator for assistance if the order was placed with a paper form.

Fetal sex and sex chromosome aneuploidy: Pregnant individuals can choose cfDNA screening but opt out of being informed about the predicted sex of the fetus. If the pregnant individual has a screen-positive result, it is likely that they will be informed of the sex of their fetus since individual SCAs are usually unique to either females or males. Pregnant individuals should be informed before screening that if they receive a screening result that shows an increased chance for SCAs, they may be told the sex of the fetus.

Fetal sex will be interpreted as:

- Consistent with female
- Consistent with male
- Fetal sex not requested
- No result on fetal sex

Always include critical prenatal screening provider information on the screening test order: Provider name, California license number, National Provider Identifier (NPI), address, zip code, phone, and fax numbers.

Collecting blood samples

Some providers collect blood samples in their office rather than sending patients to a blood collection facility. The following is how to collect blood samples for the two blood screening tests:

- MSAFP: Read the instructions on how to collect, prepare, and mail the MSAFP blood sample to the designated lab found on the CalGenetic Portal.
- cfDNA: Individual cfDNA companies provide cfDNA blood sample collection instructions through the CalGenetic Portal Resource Page. The instructions will also be available on the **PNS Program Portal Resource page** (https://bit.ly/PNSPortalResources).

RESEARCH

GDSP's California Birth Defects Monitoring Program (CBDMP) is mandated to conduct public health surveillance for chromosomal and structural anomalies. CBDMP helps researchers to identify the causes of these anomalies and other health problems. After MSAFP screening is completed, the PNS Program saves samples from certain counties and stores them. Available data includes participants demographic data, analyte values for the screening tests as performed, and determination of screen positive or screen negative status.

Using de-identified (anonymous) samples for research is very important. The use of these samples has let GDSP develop new tests. Using the information (including race/ethnicity) of California pregnancies helps researchers and allows GDSP to improve prenatal screening for all pregnant individuals in California.

Researchers must sign a "use agreement" that prevents them from using or sharing the blood sample and associated data beyond scope of the approved research project. When the research project has been completed, any remaining blood must be destroyed. It is illegal for the blood samples to be used for any other purpose.

GDSP adheres to all rules regarding human subjects' research as described by state and federal laws. The PNS Program does not store the sample to gather DNA on pregnancies. The samples are not provided to any state or national forensic DNA data banks. Participants can decline the use of their MSAFP sample for research without affecting their test results in any way.

SCREENING TEST RESULTS

Viewing screening test results on the CalGenetic Portal is faster and easier than waiting for results mailers. CalGenetic Portal users will need the PNS form number that was generated at the time of online order submission, and the patient's date of birth as submitted to the PNS Program or updated by a PNS case coordinator to view a result.

When providers must use a paper PNS Screen "Consent and Order Form," they will be unable to view patient results on the CalGenetic Portal. Results will be sent by mail within 7-10 days of sample collection. If no results are received by 10 days after blood draw for a cfDNA or MSAFP test, providers should call their regional PNS case coordinator center.

Results and interpretations for screening tests

Table 1: Findings, results, and actions authorized

Finding	Result	Action Authorized
cfDNA, Screen Positive	Increased risk for trisomy 21, trisomy 18, trisomy 13, Turner syndrome, Klinefelter syndrome, trisomy X, or XYY	Refer patient to a state-approved PDC
MSAFP, Screen Positive	Increased risk for neural tube defects	Refer patient to a state-approved PDC
cfDNA or MSAFP, Screen Negative	No increased risk for trisomy 21, trisomy 18, trisomy 13, Turner syndrome, Klinefelter syndrome, trisomy X, and XYY or neural tube defects,	No follow-up authorized by the PNS Program. Do not draw another sample.
cfDNA no call (usually due to low fetal fraction)	Sample unable to be analyzed	Contact PNS case coordinator to refer patient to a PDC or draw another cfDNA sample
Invalid screening test results (see table below for specific possible results for invalid samples)	Sample unable to be analyzed	See Table 2 on page 22

Screen Negative: No follow-up services are authorized. A negative result means that the participant's individual risk for the screened fetal chromosomal or structural anomalies is low enough that follow-up services are not covered by the PNS Program. No screening test can detect all fetal chromosomal and structural anomalies, and there is still a chance that the fetus has a chromosomal or structural anomaly. Clinicians always have the option of ordering follow-up services outside the PNS Program if patients have other financial or insurance resources.

Screen Positive: Follow-up services are authorized. A "screen positive" result indicates that the participant is at increased risk for one or more of the screened fetal chromosomal or structural anomalies, and the PNS Program will cover follow-up services as part of the prenatal screening fee. All screen-positive results are calculated for a pregnancy with a single or twin gestation.

Invalid screening test results. Sometimes the screening results are invalid for a variety of reasons. Please see Table 2 below for the possible result categories for invalid samples.

No Call Result

- Before 18 weeks 0 days, a "no call" result due to low fetal fraction would make the participant eligible for a redraw. After a second draw with a "no call" result (even if the result comes in after 21 weeks 0 days), then the participant is eligible for referral services. The eligible follow-up services for pregnant Individuals with two "no call" results for cfDNA testing are the same as a cfDNA positive result.
- After and including 18 weeks 0 days, a "no call" result due to low fetal fraction, the participant is eligible for a redraw or referral services.
- After 21 weeks 0 day, a "no call" result due to low fetal fraction makes the participant eligible for referral services.
- If the "no result" is due to an inadequate specimen for laboratory processing, it is not considered a high-risk result, and it does not count as a "no call due to low fetal fraction" result.

Inconclusive Result

- An "inconclusive result" instead of "no call result" is terminology used for SCAs. SCA "inconclusive results" will not be authorized for redraw as is a "no call result" for autosomal genetic conditions screened by the PNS Program (e.g., trisomy 21, 18, and 13).
- The reason for an SCA inconclusive result is not always due to low fetal fraction. Some laboratories that show atypical results can be contacted for further information. Empirically, inconclusive results are not as likely to resolve to a positive or negative result with repeated testing.

The pregnant individual with an inconclusive result through the PNS Program will be eligible for follow-up at a state-approved PDC. These screening results will be differentiated from atypical and indeterminate findings. The testing lab will send these findings in the lab comments. For these inconclusive results findings, the PNS Program will also authorize a referral for follow-up services at a PDC.

Low fetal fraction affecting the entire cfDNA screening panel will be eligible for a single redraw.

If the testing lab could not obtain results for trisomy 21, 18, and 13 due to low fetal fraction or other specimen inadequacy, then SCA results will not be reported. The pregnant individual may be eligible for redraw/retest.

The PNS case coordinator contacts the provider if their participant requires or is eligible for a re-draw, depending on the PNS Program's case management protocols. Providers must place a new order and print a new "Consent and Electronic Order Confirmation." The PNS Form number and sample ID barcode will be new, but the case ID for the participant will be linked to all samples. An automated computerized matching process will identify all samples for a single pregnancy.

Additional screening results correspondence

New result mailer (modified mailer): this mailer is sent to the provider whenever the screening result changes. For example, a corrected date or the date of birth of the patient can change a result from screen positive to screen negative or vice versa.

Confirmation of contact letter: the confirmation of contact letter is now a feature of the result mailer and is not separate. This letter is sent to the provider to officially document verbal or fax communication between the PNS case coordinator and the provider or their staff. For example, the provider agreed to a referral for prenatal diagnosis.

Table 2: Possible results for invalid samples

Interpretation	Cause	Action required
cfDNA, too early	Blood sample was drawn prior to 10 weeks 0 days of gestation	Draw another sample in the correct date range for cfDNA
MSAFP, too early	Blood sample drawn before 15 weeks 0 days	Draw another sample in the correct date range 15 weeks 0 days through 21 weeks 0 days
MSAFP, too late	Blood sample was drawn after 21 weeks 0 days	None. Do not draw another sample
Unexpected sample	A second sample was received after a valid sample for the same screening type	No action authorized. The results of an unauthorized screening of the same type are not statistically valid
MSAFP or cfDNA, inadequate sample	Sample could not be analyzed (i.e., hemolyzed, broken tube) or as indicated by your PNS case coordinator	Draw another sample before 21 weeks 0 days
Pregnancy not screenable	Reasons include fetal reduction, fetal demise >8 weeks of gestation, or more than two fetuses. No action authorized.	Only submit another sample if instructed by the PNS case coordinator
cfDNA, pregnancy not screenable	Fetal demise of any gestation	Sample is not valid. No action authorized. Only submit another sample if instructed by your PNS case coordinator.
Values inconsistent with pregnancy	This result indicates the analyte levels appear to be inconsistent with pregnancy	The clinician is asked to verify pregnancy status. Only submit another sample if instructed by the PNS case coordinator

Sharing test results with patients

Only a licensed health care professional should explain a patient's result to them, whether screen negative or screen positive, and should help the patient decide what action to take after a screen-positive result. Licensed health care professionals' staff who discuss screening results with patients must understand the scope and purpose of the PNS Program.

Please note that the Prenatal Screening Patient Booklet refers to screening results as "no increased chance" of a "genetic condition" or "neural tube defect" (screen negative) or an "increased chance" of a "genetic condition" or "neural tube defect" (screen positive). Invalid samples are called, "no call." These terms are appropriate to use with patients.

Sharing screen-negative results. A screen-negative result does not guarantee the fetus will not have a chromosomal or structural anomaly. No screening test is 100% accurate or screens for all chromosomal or structural anomalies.

Sharing screen-positive results. Prenatal screening tests are not diagnostic tests. They provide the risk or chance of carrying an affected fetus. A diagnostic test can give a definite answer about whether the fetus has a chromosomal or structural anomaly. A screen-positive result does not mean that there is a problem, only that there is an increased risk for a problem.

Sharing a "no-call result." There are a few possible reasons why prenatal cfDNA screening may not provide a result. It could be due to poor DNA quality, a problem with the quality of the specimen due to shipping of the sample, or a low fetal fraction. Fetal fraction represents the percentage of placental DNA that is in the pregnant individual's blood. "No-call" results can happen when there is not enough fetal DNA to examine, or the sample did not pass all the quality control steps at the lab. Many pregnant individuals will get a valid result if they have the cfDNA screening repeated with a second blood draw. The program will authorize a referral to the PDC if the first "no-call" result is associated with a gestational age that is 18 weeks 0 days or greater. If less than 18 weeks 0 days, a second specimen will be required. Another reason for a "no-call" result is for an atypical finding. In this case a result for the autosomal trisomies (trisomy 21, trisomy 18, and trisomy 13) could not be completed due to a biological condition that will persist even with a redrawn specimen. Conditions that cause an atypical result could be fetal or maternal in origin. Follow-up services for atypical findings are outside the scope of the PNS Program and are, therefore, not authorized by nor paid for by the PNS Program. However, it is recommended that these patients be referred directly for prenatal diagnosis.

Sharing an "Inconclusive Result." An inconclusive result during prenatal screening for SCAs may stem from multiple factors, including issues beyond low fetal fraction. These factors include but not limited to a demised co-twin, benign variations in the structure of the X or Y chromosome (copy number variants), and mosaicism for monosomy X or XXX in the mother or placenta. Follow-up services for inconclusive results in SCAs are authorized by the PNS Program.

Please note that as of the initial publishing of this document, SCA screening in twin gestations is very limited. Moreover, none of the state-contracted laboratories can provide SCA results when the pregnancy uses an external ovum donor as opposed to the participants own eggs.

Best practices when speaking to a patient with a screen-positive test result include the following:

- Be sensitive when speaking to the patient. Do not leave anxiety-producing news of screen-positive results on a patient's voicemail.
- Avoid calling screen-positive results late on Friday or before holidays unless you have someone available to respond to questions.
- Do not use the inappropriate term "abnormal" result. Instead, say "increased chance of a genetic condition or neural tube defect."
- Tell the patient that all follow-up services are voluntary.
- Inform them that these follow-up services are offered at no additional cost at a state-approved PDC.

FOLLOW-UP DIAGNOSTIC SERVICES

Pregnant individuals should know that a critical difference between the California PNS Program and the commercial cfDNA screening companies is the inclusion of follow-up services. If prenatal screening through the PNS Program shows an increased chance of one of the conditions screened for by the PNS program, the PNS Program offers follow-up services at no additional cost at a state-approved Prenatal Diagnosis Center (PDC). These services include genetic counseling, ultrasound exam, and diagnostic testing (chorionic villus sampling {CVS} or amniocentesis).

Follow-up services authorized by the PNS Program are only provided at state-approved PDCs. Please see the **PNS Program Information for Providers web page** (https://bit.ly/PNS4Providers) for a current list of state-approved PDCs.

When follow-up services are authorized by the PNS Program, the clinician is notified by a PNS case coordinator. The clinician should contact the patient and offer a referral to a state-approved PDC for authorized services (at no additional cost). Some follow-up services may not be available after 24 weeks gestation.

Genetic Counseling
All participants receive counseling by a state-licensed genetic counselor. The counseling includes a family history and explanations of possible reasons for a screen-positive result, as well as the risks, limitations, and benefits of diagnostic procedures.

Comprehensive Ultrasound
At state-approved PDCs, ultrasound examinations are performed by consultative sonologists. The ultrasound exam meets American College of Obstetricians and Gynecologists (ACOG), American Institute of Ultrasound in Medicine (AIUM), and American College of Radiology (ACR) standards. With services after 15 weeks, a comprehensive survey of fetal anatomy is performed to detect fetal structural anomalies.

Prenatal screening cases with gestational age dating by NT CRL, including second trimester cases, will not be re-dated after an ultrasound.

Amniocentesis
If the ultrasound findings do not explain a screen-positive result, or the findings suggest a chromosomal anomaly or open NTD, amniocentesis is usually offered to the patient. The amniotic fluid is used to determine fetal karyotype, amniotic fluid AFP levels, and the presence of acetylcholinesterase, if appropriate.

Chorionic villus sampling
CVS may be offered to the patient after screen-positive results, depending on the patient's gestational age and the availability of CVS practitioners. CVS or amniocentesis results are usually available within two weeks after the procedure. The risk of miscarriage associated with CVS or amniocentesis is less than 1% at state-approved PDCs.

UNIQUE PREGNANCIES

Is the pregnancy the result of a donated ovum?

If the pregnancy is the result of a donated ovum, indicate this on the order, including the age of the donor at time of donation. Even if the patient is using their own eggs, both the patient's current age and age at time of egg preservation should be reported. Two results will be calculated for MSAFP screening: one using the patient's date of birth and the other using the donor's age. For cfDNA screening: Fetal fraction is significantly lower in singleton IVF conceptions compared to spontaneous conceptions and is a major factor in a no call result.

Is the pregnancy at risk of Zika virus infection?

Zika virus infection in the pregnant individual can cause structural anomalies such as microcephaly in the fetus. If a pregnant individual has signs and symptoms of Zika virus disease, a travel history to an area with risk of Zika virus transmission, or a sexual partner's potential exposure, clinicians should follow guidelines in **Zika Guidance for HCPs Caring for Pregnant Individuals and Newborns**, Information for California Birthing Hospitals: Assessment and Testing for Zika Virus Infection in Pregnant Women and their Newborns.(https://cdph.ca.gov/Programs/CID/DCDC/Pages/ ZikaInformationforHealthProfession als.aspx).

Blood transfusions

A blood transfusion does not exclude a participant from cfDNA or MSAFP screening since the assay targets are specific to pregnancy. here are no restrictions on cfDNA or MSAFP screening and transfusions

WHEN TO RECOMMEND GENETIC COUNSELING

Providers should ask patients some important questions about medical history before offering prenatal screening. First, providers should review a patient's medical and family history to determine if any of the following situations apply.

- **Does the patient have a history of any of the following:**
  - Multiple miscarriages
  - Teratogen exposure
  - Suspected fetal anomaly
  - Diabetes

- **Does the patient or their partner have a family history of structural anomalies or genetic anomalies?**

- **Are they known carriers of genetic traits for conditions such as Tay-Sachs, sickle cell, or cystic fibrosis?**

Is there a family history of neural tube defects?
Has the patient taken certain teratogens (one month prior to conception or during the first trimester)? Teratogens include the following:

- Carbamazepine (Tegretol®, Carbatrol®, Atretol®),
- Valproic acid/valproate/divalproex (Depakene®, Depakote®, Stavzor®)
- Note: This is not a complete list.

What to do if family history indicates a cause for concern?

- The patient should be referred directly to a genetic counselor For genetic counseling. This pre-screening-test genetic counseling is not covered by the PNS Program. Many health plans and Medi-Cal cover genetic counseling for these indications.
- Do not order prenatal screening if the patient is scheduled for or has already had amniocentesis.

Important Note: The PNS Program will pay for follow-up services only if the prenatal screening result provided by the PNS Program is screen positive, a no-call cfDNA result, or an indication of a fetal abnormality.

Large Nuchal Translucency

The PNS Program is no longer collecting nuchal translucency (NT) measurements and will no longer refer participants to a state-approved PDCs solely based on a large NT measurement. ACOG and American College of Medical Genetics and Genomics (ACMG) guidelines recommend that patients with a 3.0 mm or larger NT measurement should be referred directly for prenatal diagnosis.

SOME PREGNANCIES ARE NOT ELIGIBLE FOR SCREENING

Fetal reduction. Do not order cfDNA or MSAFP screenings for individuals who have undergone procedures to reduce the number of fetuses. Pregnancies with terminated fetuses usually have very high serum MSAFP levels and cfDNA may persist in the blood for a long time after fetal reduction. Rapid increases in the fetal fractions in the deceased cotwin can continue until 7–9 weeks after reduction.[3] This prevents an accurate risk assessment. If a blood sample is submitted, the results are considered invalid, and no follow-up services are authorized.

Fetal loss. Fetal demise (e.g., demise of one fetus of twin pregnancy, vanishing twin, molar pregnancy, fetal pole no longer present or ectopic fetus) is an exclusionary factor for cfDNA screening.[3] In the case of cfDNA screening, if the PNS Program uncovers a fetal demise at any gestation, the results will be considered invalid if the patient has already undergone screening. For MSAFP screening, a fetal loss after 8 weeks 0 days gestation makes the pregnancy ineligible for screening.

Diagnosis of a fetal chromosomal or structural anomaly. If a patient had diagnostic testing or has a positive test for fetal anomaly, they are not eligible for cfDNA or MSAFP screening through the PNS Program. If a patient has a structural anomaly, they are not a good candidate for the PNS Program. The patient should be advised to go for diagnostic testing.

Solid organ transplant for the pregnant individual. It is possible that small fragment DNA originating from the transplanted organ and used to test for common autosomal aneuploidy (trisomy 13, 18, 21, Turner syndrome, Klinefelter syndrome, trisomy X, and XYY)) may contribute to abnormal results when diagnostic testing finds no chromosomal anomaly. Fetal sex discrepancy after cfDNA screening compared with sonographic findings has been reported due to the contribution of DNA from a donor solid organ and marrow transplant.[1]

Multiple gestation of three or more fetuses. Triplets or higher order gestation pregnancies are an exclusion factor for both cfDNA and MSAFP screening. Do not order prenatal screening for individuals who are carrying three or more fetuses. If a blood sample is submitted, the results are considered invalid, and no follow-up services are authorized.

Malignancy. The presence of a malignancy in the pregnant individual is an exclusion factor for cfDNA screening. Nearly all cancers have genetic changes that can be found in cfDNA screening. A high prevalence of cancer is seen in pregnant individuals with more than one aneuploidy detected through cfDNA screening contradictory to fetal karyotype.[4]

[3] Wiles, KS, Tillett, AL, Harding, KR, Solid organ transplantation in pregnancy. The Obstetrician & Gynaecologist. 2016;18: 189-197Min Chen,Fengxia Su,Jiayan Wang, et al. Temporal persistence of residual fetal cell-free DNA from a deceased cotwin after selective fetal reduction in dichorionic diamniotic twin pregnancies. Prenatal Diagnosis. 2021Nov;41(12):1602-1610

[4] Bianchi DW, Chudova D, Sehnert AJ, et al. Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies. JAMA. 2015;314(2):162–169.

SCA and twin gestations

SCA detection via cfDNA screening in the California PNS Program is only available for monozygotic twins. SCA screening is not available for multiple gestation of two (dizygotic twins) or more fetuses. Maternal plasma analyzed with cfDNA has shown that the circulating fetal DNA from both placentas can vary leading to inaccurate results. Dizygotic twin pregnancies may proceed through the PNS Program but can only receive results for T21, T18, T13, and fetal sex. If it's unclear if the twins are dizygotic or monozygotic, the cfDNA laboratory may be able to determine the zygosity of the pregnancy.

Several instances will invalidate the cfDNA result, including previous positive, negative, or atypical cfDNA screening result. Screen-positive results outside of the PNS Program do not make a patient eligible for follow-up services with the PNS Program. However, if a patient had a for the PNS Program, as long as they meet all other eligibility screening criteria. An atypical finding causes a cfDNA test failure that could not produce a valid result due to a biological condition that will persist even with a redrawn specimen. Conditions that cause an atypical results could be fetal or maternal in origin. These patients should be referred directly for prenatal diagnosis outside of the PNS Program.

ULTRASOUND AND PREGNANCY DATING

Providers should conduct a first trimester ultrasound for gestational dating and to rule out major structural anomalies like a large NT, heart defects, cleft lip, and spina bifida. This list is not a complete list. If structural anomalies are found, the provider may want to discuss with the patient diagnostic testing at a state-approved PDC for follow-up services instead of prenatal screening.

Dating with ultrasound (US) available: Although the PNS Program is no longer accepting NT measurements, gestational age by NT CRL is the best dating method for screening when the NT ultrasound is done by a credentialed NT practitioner. If NT ultrasound dating is not available, a transvaginal or transabdominal US can be used as a method of dating for prenatal screening.

Dating with last menstrual period (LMP): If no ultrasound dating is available, LMP can be used as a method of dating for prenatal screening.

Exam dating: Exam dating is the least reliable dating for screening.

Estimated due date calculator (EDD): The PNS Program has an online calculator available to clinicians at no cost to calculate a patient's gestational age and estimated due date (EDD). Find the calculator on the **gestational age calculator** (https://calgenetic.cdph.ca.gov/content/gdsp/en/portal-landing-page.html). This is only used for cfDNA screening. The EDD value will be calculated for orders placed on the online CalGenetic Portal automatically based on US, LMP, or PE dating entered, but EDD must be provided and written in for orders using "Consent and Order Form" PDFs.

Corrections and updates to pregnancy dating: An individual might have different results for NTDs whether US, LMP, or PE dating is used, even if the gestational ages are the same by all methods. Redating an LMP-dated or physical exam-dated pregnancy by ultrasound or NT CRL may significantly change an individual's trisomy 21 risk estimate, and the new estimate provides a better risk assessment.

cfDNA screening may be affected if redating makes the sample receipt too early.

If ultrasound dating information becomes available after the prenatal screening sample has been submitted, providers are encouraged to call the PNS case coordinator's phone number printed on the results mailer and request that a new screening result be issued using the new dating.

Dating with twins: The CRL and second trimester fetal biometry from the larger fetus are most accurate in determining the estimated due date for a twin gestation by ultrasound.[5, 6] If the woman presents after 14 weeks' gestation, the larger head circumference should be used. Twin pregnancies should be dated when CRL measurement is between 45 and 84 mm (11 weeks, 0 days to 13 weeks 6 days of gestation).

Sexual Orientation and Gender Identity Information (SOGI)

Effective July 1, 2018, the PNS Program must comply with Government Code Section 8310.8. Section 8310.8 states that all California state agencies collecting personal information must also include a questionnaire about sexual orientation and gender identity.

Remind the participant that the Prenatal Screening Patient Booklet contains a URL and QR code on Page 15 that links to the PNS Program's **Sexual Orientation and Gender Identity (SOGI) information survey** (https://forms.office.com/g/LRUWGVE7Xx).

Advise the participant that submission of this information is voluntary and will not affect any prenatal risk assessment or results interpretation. This information is collected in aggregate and will not be traceable to individual participants and/or prenatal screening test results. Participants only need to submit a survey once per pregnancy.

Link to Patient SOGI Survey

REPORTING FETAL CHROMOSOMAL AND STRUCTURAL ANOMALIES

Reporting neural tube defects

State regulations (CCR, Title 17, Sections 6531 and 6532) require that NTDs and/or chromosomal anomalies found in fetuses or infants less than one year of age be reported to CDPH's Genetic Disease Screening Program (GDSP). All cases of NTDs and chromosomal anomalies must be reported, even if the patient did not have prenatal screening or if the screening result was negative.

The report should be made within 30 calendar days of the initial diagnosis on the form, "A Confidential Case Report of a NTD in a Fetus or an Infant Less than One Year of Age," provided by GDSP. Call a PNS case coordinator to ask questions.

[3] Tracey H. DeYoung, Sharon K. Stortz, Garrett M. Wren et al. 385: What is the optimal strategy to date a twin gestation by ultrasound? AJOG, American Journal of Obstetrics and Gynecology. 2019 Jan 220;1 SUPPLEMENT, S263

[4] R Townsend and A Khalil. Ultrasound surveillance in twin pregnancy: An update for practitioners, Ultrasound. 2018 Nov; 26(4): 193–205.

Reporting chromosomal anomalies

State regulations (CCR, Title 17, Section 6532) require the reporting of all cases of Down syndrome or other chromosomal anomalies in a fetus, or an infant under one year of age. California cytogenetic laboratories are responsible for this reporting. However, clinicians become responsible for this reporting if they send the sample to a laboratory outside of California.

The report should be made within 30 calendar days of the initial diagnosis on the form, "A Confidential Case Report of a Chromosomal Defect in a Fetus, or an Infant Less Than One Year of Age," provided by GDSP. Call a PNS case coordinator to ask questions.

Registry reporting form is available online (www.cdph.ca.gov/Programs/CFH/DGDS/Pages/pde/Registry-Reporting-Forms.aspx).

Reporting outcomes of pregnancy

The California Code of Regulations (Title 17, Section 6527) requires prenatal screening providers to fill out a Confidential Report of an Outcome of Pregnancy (CDPH 4452 form) for the ongoing analysis of the effectiveness of the policies and practices adopted by the California Prenatal Screening Program. We request that the confidential report be completed for screened individuals who had a screen-positive prenatal screening test result, who have a pregnancy with a twin gestation, or who were selected into a comparison group with screen-negative test results. The confidential report is automatically sent to the provider who ordered the prenatal screening test approximately 60 days after the patient's due date. Providers should complete the form online via the secure **CalGenetic Portal** (https://calgenetic.cdph.ca.gov/) as soon as possible. If a confidential report is not submitted online within a period of 60 days after the initial request, a second request will be sent to the provider. Please email us at **CDPH_GDSP_PNS_Outcomes@cdph.ca.gov** for any questions or requests.

Table 3: Midtrimester Risk for Chromosome Abnormalities by Maternal Age at Term

Maternal Age	Risk for Trisomy 21 [7,8]	Risk for Trisomy 18 [1,2]	Risk for Trisomy 13[9]
20	1:1140	1:4430	*
21	1:1130	1:4380	*
22	1:1110	1:4320	*
23	1:1090	1:4250	*
24	1:1060	1:4150	*
25	1:1030	1:4020	*
26	1:990	1:3860	*
27	1:940	1:3660	*
28	1:880	1:3420	*
29	1:810	1:3140	*
30	1:720	1:2820	*
31	1:630	1:2460	*
32	1:540	1:2090	1:6667
33	1:441	1:1720	1:6667
34	1:351	1:1370	1:5000
35	1:272	1:1060	1:4000
36	1:205	1:800	1:3333
37	1:153	1:600	1:2857
38	1:114	1:444	1:2000
39	1:85	1:333	1:1667
40	1:65	1:255	1:1250
41	1:51	1:200	1:1000
42	1:42	1:162	1:800
43	1:35	1:136	1:606
44	1:30	1:117	1:465
45	1:27	1:104	1:357
46	1:24	1:94	1:278
47	1:22	1:87	1:215
48	1:21	1:82	1:167
49	1:20	1:79	1:128
50	1:19	1:76	*

Table 3: Midtrimester Risk for Chromosome Abnormalities by Maternal Age at Term (continued)

The numbers in this table are approximate risks based on data currently available. These numbers are population- based risk estimates and should not be presented as an individual's specific risk.

These numbers represent the estimated risk for a fetus with Down syndrome, trisomy 18 and trisomy 13 at midtrimester. Approximately 23% of Down syndrome fetuses and 70% of trisomy 18 fetuses will be lost between midtrimester and term.

[5] Morris JK et al.: Revised estimates of the maternal age specific live birth prevalence of Down's syndrome.
J Med 2002; 9:2-6

[6] Hook, KB: Chromosome abnormalities and spontaneous fetal deaths following amniocentesis; further data and associations with maternal age Am J Hum Genet 1983 35:110- 116.

[7] Hook, KB, Cross, PK, Schrelnemachers, DM.: Chromosomal abnormality rates at amniocentesis and live-born Infants J Am Med Assoc 1983, 249: 2034-2038

* Data not available

Table 4: Acronyms/Abbreviations

Acronym/Abbreviation		Definition
ACMG	American College of Medical Genetics	An organization composed of health care professionals committed to the practice of medical genetics and genomics
ACR	American College of Radiology	A professional medical society representing nearly 40,000 diagnostic radiologists
ACOG	American College of Obstetricians and Gynecologists	The premier professional membership organization for obstetrician–gynecologists
AIUM	American Institute of Ultrasound in Medicine	A multidisciplinary association dedicated to advancing the use of ultrasound in medicine
AWD	Abdominal wall defect	A type of structural anomaly affecting fetal abdomen development
BPD	Biparietal diameter	Type of ultrasound measurement used for gestational dating
cfDNA	Cell-free DNA	Circulating fragments of fetal DNA that can be found in a pregnant individual's blood during pregnancy
CRL	Crown-rump length	Type of ultrasound measurement used for gestational dating
CVS	Chorionic villus sampling	Diagnostic testing for first trimester screenpositive chromosome abnormalities
FMF	Fetal Medicine Foundation	Educational and quality review program for fetal medicine. Provides credentialing for nuchal translucency practitioners
GDSP	Genetic Disease ScreeningProgram	State program providing oversight of California Prenatal Screening (PNS) Program
GLS	General Logistics Systems	Courier service for PNS Program
LMP	Last Menstrual period	Dating of gestational age based on date of last expected menses
MSAFP	Maternal Serum Alpha-Fetoprotein	Analyte used in second trimester screening for structural anomalies

Table 4: Acronym/Abbreviation (continued)

Acronym/Abbreviation		Definition
NT	Nuchal Translucency	An ultrasound measurement of the fluid filled area behind the fetal neck used for prenatal screening of chromosomal abnormalities
PDC	Prenatal Diagnosis Center	State-approved prenatal diagnosis centers
NTD	Neural Tube Defect	A structural anomaly affecting fetal spine and brain development
SCA	Sex chromosome aneuploidies	Genetic conditions resulting in an extra or missing copy of the sex chromosomes, X and/or Y
T21	Trisomy 21, Down syndrome	A genetic condition resulting in an extra copy of chromosome 21
T18	Trisomy 18, Edwards syndrome	A genetic condition resulting in an extra copy of chromosome 18
T13	Trisomy 13, Patau Syndrome	A genetic condition resulting in an extra copy of chromosome 13

Prenatal Screening Provider Handbook Appendices

The appendices can be accessed on the **PNS Program Information for Providers web page** (https://bit.ly/PNS4Providers)

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California Prenatal Screening Program

www.cdph.ca.gov/PNS

California Department of Public Health
Prenatal Screening Program
Genetic Disease Screening Program
850 Marina Bay Parkway, F175
Richmond, CA 94804
pns@cdph.ca.gov
866-718-7915 toll free

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