

How the Pink or Blue® DNA Gender Test Works

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After finding out you are pregnant, have you ever wanted to know your baby's gender so that you can begin to build a strong parental bond and more vividly imagine your new life with her or him? Studies have shown that mother's and baby's cells can pass between their bodies to help fight disease, suggesting that maternal bonding is physical as well as mental. Upon hearing the words "I am pregnant", many parents immediately begin to wonder whether they are going to have a boy or girl. Here we describe the Pink or Blue® DNA based gender test and provide details about the scientific methodology used to achieve an accuracy of greater than 95% (based on our internal quality improvement study). Our mission is to provide you with information that you can use to bond earlier with your baby.

Pink or Blue® is a direct-to-consumer product that does not require Clinical Laboratory Improvement Amendments (CLIA) certification or Food and Drug Administration (FDA) clearance. The FDA does not regulate the test because the test is classified as non-medical and not marketed as a tool for medical diagnoses. However, Consumer Genetics is in the process of obtaining both International Organization for Standardization (ISO) 9001 (a quality management standard) and CLIA accreditation



because the company believes that a good quality system will foster more efficient business practices.

Pink or Blue® is a unique DNA-based test that tells you whether you are having a boy or girl at 10 weeks after the start of your last menstrual period, approximately 7 weeks post-conception. This service provides information that has no impact on the health of the mother or the baby. Consumer Genetics, Inc. makes no medical diagnoses, recommendations, treatments, or medical or health claims with this test.

Current medical procedures like amniocentesis, chorionic villus sampling (CVS), and late term ultrasound are also used for gender determination but these procedures are done for diagnostic reasons only. All of these clinical methods pose some risk to the baby. The chart on the right helps depict the timeframe that these tests may be done.

Amniocentesis can be performed as early as 9 weeks and is associated with a small risk of miscarriage and other pregnancy complications (infection, premature labor, injury to the fetus, cord, or placenta, rupture of membranes, etc.). The test takes about 2-4 weeks to process and sex determination is close to 99%. CVS is usually performed between 8 and 11 weeks gestation

and is generally provided to women who have a high risk of genetic abnormalities. Test results are available within 7 to 14 days and the test has a small risk of miscarriage. Recent studies indicate that if the CVS is done before 10 weeks gestation there is an added risk of limb deformity and a serious decline in amniotic fluid is possible. There is also a greater incidence of false positives because of the differences between the cellular genetic material of the chorionic villi and the fetus. Sex determination is close to 99% accurate.

A myriad of publications describe how ultrasound can be used to determine gender. Some publications state accuracy at 80 to 95% during weeks 13 to 17 but most clinicians do not check for gender until the formal ultrasound is done. This is usually done in the late second trimester or third trimester. Accuracy at this stage is usually around 97% depending on which publications are used as references, as well as the skill of the ultrasonographer.¹

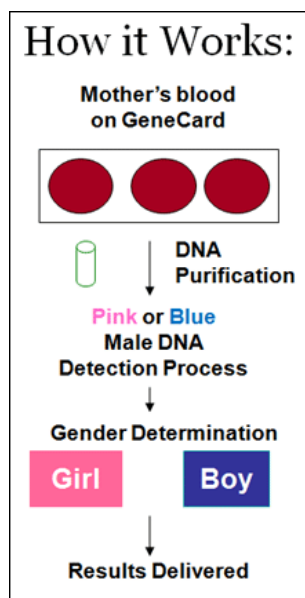
Other tests that claim baby gender determination utilize urine in much the same way that drug tests or pregnancy tests do and claim an accuracy of about 82%, but no scientific literature supports this method.² These tests can be done completely at home

# of weeks after conception date.	Probable Date of Conception	First Day of Last Period		
				Week 1
			Week 2	
			Week 3	
1			Week 4	
2			Week 5	
3			Week 6	
4	Positive Pregnancy Test		Week 7	
5			Week 8	
6			Week 9	
7			Week 10	
8	Pink or Blue DNA Gender Testing Eligibility Period		Week 11	
9			Week 12	
10		CVS		Week 13
11				Week 14
12				Week 15
13				Week 16
14		Amnio		Week 17
15				Week 18
16			Week 19	
17			Week 20	
18	Formal Ultrasound			Week 21
19				Week 22
20			Week 23	

but customers have a very large number of incorrect results.

How does the Pink or Blue® DNA Test Work?

It has been known for over ten years that the blood of pregnant women contains small amounts of fetal DNA³. DNA stands for deoxyribonucleic acid and it contains the genetic information that determines the uniqueness of a person. Most of our DNA resides inside the nucleus of our cells in threadlike bodies called chromosomes. In humans, there are 46 chromosomes, two of which are the X and Y sex chromosomes (remember XY is a boy and XX is a girl). In the 1990s, a very rapid, robust, sensitive, and



specific method, based on the speedy amplification of segments of DNA molecules by the polymerase chain reaction (PCR) technique, was introduced to type DNA. PCR enables researchers to produce millions of copies of a specific DNA sequence from a small amount of starting material. Since Y chromosomal DNA is required for us to have a baby boy, our test looks for Y chromosomal DNA by attempting to amplify this signal (create millions of copies of this specific

DNA). Finding small amounts of fetal Y DNA is very difficult and multiple controls are in place to insure quality results.

The classic study validating the process of detecting Y chromosomal DNA in the maternal blood in a clinical setting was published in the Journal of Human Genetics in 2005⁴. Galbiati et. al. obtained blood from 1,837 pregnant women at different stages of pregnancy and isolated fetal DNA as early as 5 weeks of pregnancy. This included women with previous pregnancies, miscarriages and live births. The isolated fetal DNA was amplified and analyzed using PCR. The test results were confirmed after the baby's birth and their study had a 99% accuracy rate at correctly predicting gender. Note: *The 99% accuracy rate in the Galbiati research study was performed under controlled conditions and with much larger blood samples. The Pink or Blue® test accuracy rate in the hands of the consumer is calculated to be greater than 95%, based on our internal research described below.*

The Pink or Blue® test is highly accurate in determining the presence of male DNA in the mother's blood. However, there are a few instances, such as a recent blood transfusion, multiple-child pregnancies or a bone marrow transplant, which may also contribute to the presence of male specific DNA in the maternal blood. Although unlikely and extremely rare, these instances do occur, and may lead to false-positive results.

We suggest that you do **NOT** take the Pink or Blue® test if any of the following conditions are true:

- You had a miscarriage or termination within three months of your current pregnancy
- You have had a bone marrow transplant from a male in your lifetime
- You have had a blood transfusion within the last 18 months
- You are taking medication to treat blood-related conditions and/or undergoing anti-coagulant therapy.

Our patent pending technology utilizes the natural process of DNA movement from the baby to the mother's blood in order to accurately predict the baby's gender. Our test has been used to determine gender in more than 6,000 women as early as 7-weeks post-conception. We are able to do this by purifying the blood sample on our gene card so that we can extract the DNA and then attempt to amplify the fetal Y chromosomal DNA. Unlike Galbiati's methods, we do not require a blood draw and use approximately 100 to 1000 times less blood. Lack of fetal Y chromosomal DNA indicates a girl whereas presence of Y chromosomal DNA indicates a boy.

The Pink or Blue® test is easy to complete in the comfort of your own home. The kit consists of alcohol swabs to clean the finger, lancets to prick the finger, and a gene card for collection of the drops of blood. The gene card and a consent form are mailed back to our laboratory for processing. We need the information on this form to help make our gender determination as accurate as possible.

Our technology is more sensitive than previous techniques found in the literature and thus can provide an accurate answer from just three drops of maternal blood (average amount of drops to fill one circle on the gene card). Although results can be obtained from this small amount, we request that the expectant mother fill all three circles so that we have redundant information at hand in cases where there are initial inconclusive results. We always test the DNA sample twice, in parallel, for quality assurance

and reliability purposes. Male-specific DNA will be present in the specimen if the baby is a boy.

How We Determine our Accuracy

We have developed a quality improvement methodology to assess our accuracy and we use this to improve our processes with the goal of reaching 99% accuracy. We realize that our customers would like an unbiased study to validate our claims and we are in the process of conducting two clinical studies that we will share with our customers once they are completed. Both of these studies are being conducted in California. In the meantime, we will share with you how we determine our internal laboratory gender determination accuracy.

Sample selection

Our samples consist of maternal blood spotted onto our gene card. The gene card polymerizes the blood so that it is safe to handle and mail. Once obtained in our lab, the gene card paper is cut into pieces and purified. 31 customer gene cards received in the laboratory between September 2007 and March 2008 were selected for this internal study. Samples were chosen based on the following criteria 1) blood was collected at least 7 weeks post conception as calculated using the Consumer Genetics, Inc. online pregnancy calculator, 2) a boy or girl result was released to the customer, 3) the result was confirmed by ultrasound, CVS, amniocentesis, or birth certificate (we depend on our customers to be truthful when filling out our survey, however this may not always be the case) and 4) enough gene card remained for the quality control test.

The Basic Procedure

DNA is removed from the gene card and then separated from any cell debris, protein, and RNA that may be present. This purified DNA is ready for amplification using the PCR technique. Using PCR we



look for Y chromosomal DNA. If PCR amplification occurs because Y chromosomal DNA is detected, the sample is a boy, if no amplification occurs the sample is a girl.

What are in the Controls?

DNA Extraction and purification controls include one female control purified in parallel with a batch of ten customer samples. The female control is used to set the background signal of the amplification process (remember, we are only trying to amplify the fetal male DNA so the female DNA is background).

Controls for individual samples include contamination controls; DNA extraction and purification yield controls; and an inhibition control. We have developed several methods to ascertain the presence of adult male DNA. The presence of adult male DNA will contaminate the sample and make it much more difficult to

amplify the small amount of fetal male DNA. We specifically look for certain segments of the adult Y chromosome that are different from the fetal Y chromosome. If these are found then we know contamination has occurred. These results are 100%. Our inhibition test utilizes a small amount of known adult male DNA that is added to

the sample. The inhibition control should exhibit amplification since we are manually adding our target, the Y chromosomal DNA. If no amplification is detected, contaminants must be present in the reaction which inhibits the process of making more DNA.

Analysis

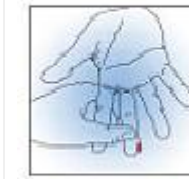
The PCR machine outputs numbers indicating how much Y chromosomal DNA was present in the purified DNA sample. These data values are analyzed as to how they fall between two threshold levels (upper and lower). Data values that are on this threshold or in between are inconclusive. The region between the upper and lower levels is large to ensure the most accurate results for our customers. This is an important point.

In the case of an inconclusive result, the sample is reprocessed if enough remains. Examination of these

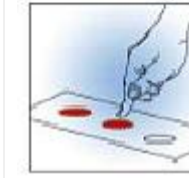
3-Steps to Collecting Specimens



1. Prick your finger using the lancets supplied in the Pink or Blue DNA Gender Testing kit.



2. Massage the pricked finger until a drop of blood appears.



2. Blot the blood onto the DNA preservation card included in the kit. Samples are sent back to the Lab for testing.

new data values with respect to the threshold will usually provide us with a confident gender determination. If none of these purifications can provide substantive data for determination, we request a new sample from the customer. If they chose to send us a new sample, results are usually clearer since there is a larger amount of fetal DNA in the woman as she progresses in her pregnancy (thus the signal is much more pronounced if a boy is present).

We do not consider inconclusive results as incorrect. Inconclusive results are discarded since re-tests are performed. Correct and incorrect results are defined as our result (boy or girl) matched the survey information or it did not match, respectively.

Results

Our survey used 31 customer samples after excluding samples that did not pass the controls. Only the first purification (that passed the individual sample controls) was considered. There were 4 inconclusive results for an inconclusive rate of 12.9%. Samples that fell in the inconclusive range were excluded since normally two purifications are completed for each sample and in this study data is from a single purification. This left 26 correct results out of 27 total results for an accuracy rate of 96.3%. There were 12 correct girls, 14 correct boys, one false positive, and zero false negatives. The specificity was 92.3% and the sensitivity was 100%.



Discussion:

Our results help show areas where factors that affect accuracy can be minimized:

- Not following the directions carefully: improperly handling kit components, improperly washing hands and not sanitizing the collection area
- Contaminating the sample: allowing males to handle kit contents or assisting with sample collection.
- Taking the test earlier than 7 weeks post-conception
- Having a recent transfusion, miscarriage or abortion
- Providing an insufficient blood sample
- Leaving the sample in a hot environment

Our results also help show areas where factors that affect accuracy can not be controlled:

- Having naturally low levels of fetal DNA during early pregnancy will not provide enough DNA to obtain a clear signal.
- Vanishing Twin: some pregnancies begin with more than one fetus but very early in the pregnancy, one of the fetuses terminates. In many cases, this occurs without the woman's knowledge. If the "vanishing twin" is male and the remaining fetus is female, the results of Pink or Blue® may be incorrect, however, with our current quality control procedures, this is very rare.
- Previous male pregnancy: in very rare cases, fetal cells may remain in the mother's body several years after birth. Our technology can detect some of this DNA as a form of contamination; however, there may be cases where the accuracy of the test may be affected by this rare occurrence.
- Having a bone marrow transplant from a male donor, blood transfusions, miscarriages or abortions: our technology can detect DNA from previous male pregnancies or from male blood/marrow donors as contamination in most cases. However, there may be instances where the accuracy of our test is affected by any of these conditions.

Conclusions:

The accuracy of our methodology based on this random subset of customers is 96.3%. Consumer Genetics is continually striving to monitor and improve the accuracy of the Pink or Blue® early gender test. Our goal is to maintain greater than 95% accuracy. Some ways to improve the test include development of a more robust method for detecting contamination and implementing even more rigorous quality control methods in the laboratory to keep reagents and equipment in consistent working order.

¹ MICHAILIDIS, GD et. al., "The use of three-dimensional ultrasound for fetal gender determination in the first trimester", *The British Journal of Radiology*, 76 (2003), 448-451, 2003

² Intelligender website, FAQ, <http://www.intelligender.com/pages.php?pageid=6#3>, October 2008

³ PCR detection of cell-free circulating fetal DNA in maternal plasma and serum (*Lo et al. Lancet 1997; 350:485-7*), Earliest detection of fetal DNA: 5 weeks of gestation (*Prenatal Diagnosis, 2003; 23: 1042*), Source of fetal DNA? Passive diffusion across placental barrier (*van Wijk et al. Clinical Chemistry, 2000; 46:729-731*)

⁴ Galbiati S, Smid M, Gambini D, Ferrari A, Restagno G, Viora E, Campogrande M, Bastronero S, Pagliano M, Calza S, Ferrari M, Cresmonesi L., Fetal DNA detection in maternal plasma throughout gestation. *Hum Genet.* 2005, 117(2-3): 243-248.