Genetics 101 for BRCA and Non-Invasive Prenatal Testing:

Presented by:
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September 28, 2015

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Presentation Agenda

- Cancer Genetics 101
- "Red Flags" for hereditary cancer
- *BRCA1* and *BRCA2* genes
  - Cancer risks, testing criteria, and management guidelines
- Additional hereditary breast cancer genes
  - Cancer risks, testing criteria, and management guidelines
- Noninvasive prenatal screening (NIPS)
  - Chromosomal abnormalities
  - Evolution of prenatal screening
  - Clinical utility of NIPS
- Genomic Client Services

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Cancer Genetics 101

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How Does Cancer Develop?

- Sporadic cancer
  - Sporadic cancer develops due to genetic changes within the cells of a specific body tissue
  - Causes for sporadic cancer include aging, hormone history, radiation exposure, smoking, alcohol use, BMI
    - **Most contributing factors are still unknown**
- Hereditary cancer
  - Hereditary cancer results from a mutation in a gene
  - Gene mutations can be passed down in families, causing an increased susceptibility to cancer
- All cancer is **genetic**, but most cancers are not **hereditary**

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How Does Cancer Develop?

Hereditary

At Birth

Sporadic

At Birth

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Examples of Genetic Variation

Color ➡ *Colour*

- *changed spelling but not meaning of word*
- *benign polymorphism*

Hear ➡ *Ear*

- *letter deletion changes the word*
- *pathogenic mutation*

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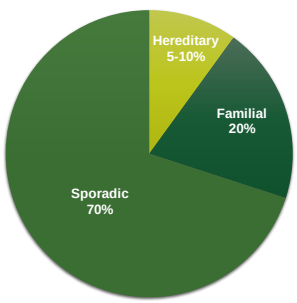
What Percentage of Cancer is Hereditary?



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"Red Flags" for Inherited Susceptibility to Cancer

- Cancer in 2 or more closely related relatives
- Multiple generations affected
- Early age at diagnosis
- Multiple primary tumors
- Bilateral or rare cancers
- Constellation of tumors consistent with a specific cancer syndrome
- Certain ethnic backgrounds (e.g. Ashkenazi Jewish ancestry)

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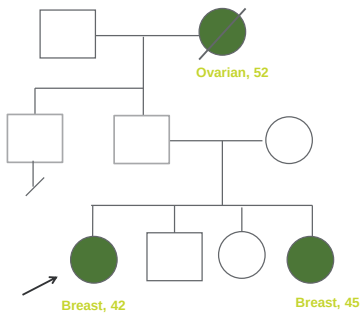
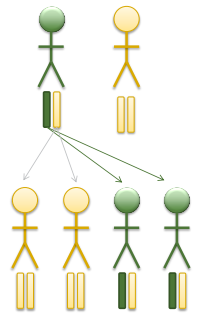
Assessing Your Patient's Family History



Autosomal Dominant Inheritance



Genetic Testing Strategy

- The right person
  - When possible, testing should begin with the individual most likely to test positive (family member who has actually had cancer, youngest age at diagnosis, bilateral disease, multiple primary cancers)
- The right test
  - Consider:
    - Is there a known familial mutation?
    - Is the patient of Ashkenazi Jewish ancestry?
    - What types of cancers have been diagnosed in the patient and/or the family?
  - Single gene(s) vs targeted panel vs large panel
- The right time
  - Are the test results needed for surgical decision making?
  - Consider the psychosocial implications of genetic testing
  - Testing is generally not warranted for individuals under the age of 18

Hereditary Breast and Ovarian Cancer Syndrome

BRCA1 and *BRCA2*

BRCA1 and *BRCA2*

The most common high-risk hereditary breast cancer genes

- Autosomal dominant
- Carrier frequency ~1/400 in general population
- Carrier frequency ~1/40 in Ashkenazi Jewish population

Cancer Risks to age 70

	BRCA1	*BRCA2*
Female Breast Cancer	55% to 65%	45% to 47%
Ovarian Cancer	39%	11% to 17%
Male Breast Cancer	1.2%	6.8%

***Also, increased risks for prostate cancer, melanoma, and pancreatic cancer**

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Medical Management Guidelines: *BRCA* positive

Cancer	Intervention	Age and Frequency
Breast	Screening	Clinical breast exam every 6-12 months, starting at age 25 y for women and 35 y for men Annual breast MRI, starting at age 25 y for women, add annual mammogram at age 30; consider baseline mammogram at 40 y for men
	Chemoprevention	(i.e. Tamoxifen, Raloxifene, aromatase inhibitors)
	Surgery	Discuss risk-reducing mastectomy
Ovarian	Surgery	Risk-reducing bilateral salpingo-oophorectomy, ideally between the ages of 35 and 40 y
	Chemoprevention	(i.e. oral contraceptives)
	Screening	Transvaginal ultrasound and CA-125 blood test; repeat every 6 months starting at age 30-35 y*
Prostate	Screening	Starting at age 40 y for *BRCA2* carriers / consider for *BRCA1* carriers
Melanoma	Screening	No specific guidelines: Consider annual dermatological examinations
Pancreatic	Screening	No specific guidelines: Consider pancreatic cancer screening if + family history

***Ovarian cancer screening has not been shown to be sufficiently sensitive or specific to support routine screening recommendation but can be considered at the clinician's discretion**

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Testing Criteria for *BRCA1* and *BRCA2*

Family History of Breast Cancer

- Relative with a previously identified *BRCA1* or *BRCA2* mutation
- 1st/2nd-degree blood relative who meets any criteria in the Personal History sections
- 3rd-degree relative with breast[a] and/or ovarian[b] cancer and ≥2 close blood relatives[c] with breast and/or ovarian[b] cancer

Personal History of Other (Nonbreast) Cancers

- Epithelial ovarian cancer[b]
- Pancreatic or prostate cancer with ≥2[d] close blood relatives[c] diagnosed with breast, ovarian,[b] pancreatic, or prostate cancer (Gleason score ≥7)

Personal History of Breast Cancer[a]

Age at Diagnosis	Additional Criteria Only 1 of the following is necessary.
≤45 y	• No additional criteria necessary
≤50 y	• ≥2 primary breast tumors[a] • ≥1 close blood relative[c] with breast cancer • Limited family history
≤60 y	• Breast cancer that is negative for ER, PR, and HER2 (triple negative)
Any age	• Patient is male • ≥1 close blood relative[c] with breast cancer diagnosed by age 50 or with epithelial ovarian[b] cancer diagnosed at any age • ≥2 close blood relatives[c] with breast cancer • ≥2 close blood relatives[c] with prostate cancer (Gleason score ≥7) or pancreatic cancer • ≥1 close male blood relative[c] with breast cancer • Ethnicity (eg, Ashkenazi Jewish) associated with higher mutation frequency

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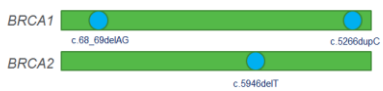
Genetic Testing Strategy – *BRCA1/BRCA2*

BRCAvantage™, Comprehensive (includes large rearrangements)

BRCAvantage™, Ashkenazi Jewish Screen



BRCAvantage™, Single Site *

* Except for patients of Ashkenazi Jewish ancestry

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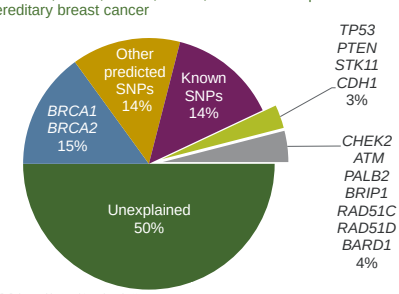
Beyond *BRCA*:

Additional Breast Cancer Susceptibility Genes

Beyond *BRCA*: Other genes

Mutations in *BRCA1* and *BRCA2* explain 15%-20% of hereditary breast cancer cases

Mutations in *TP53, PTEN, STK11, CDH1,* and *PALB2* explain an additional 3% – 4.5% of hereditary breast cancer



Couch *et al.*, *Science* **343** (6178): 1466-1470

Beyond *BRCA*: Gene Panels

BRCAvantage Plus™

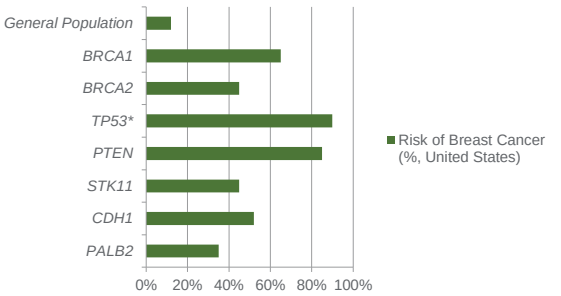
BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, PALB2

- Simultaneous analysis of seven genes associated with hereditary breast and other cancers
- These genes all have NCCN guidelines for medical management if results are positive
- More efficient and cost-effective if personal/family history raises concern for more than one syndrome
- May be useful for those who have previously tested negative for a single syndrome, but whose personal or family history remains strongly suggestive of an inherited susceptibility
- Also available as a reflex test if *BRCA* analysis is negative

BRCAvantage Plus™

	Gene						
Associated Cancer	***BRCA1***	***BRCA2***	***TP53***	***PTEN***	***CDH1***	***STK11***	***PALB2***
Breast	•	•	•	•	•	•	•
Colorectal			•	•	•	•	
Endometrial			•	•		•	
Stomach			•		•	•	
Melanoma	•	•	•	•			
Ovarian	•	•	•			•	
Pancreatic	•	•	•			•	•
Prostate	•	•	•				
Other			•	•		•	

Breast Cancer Risks

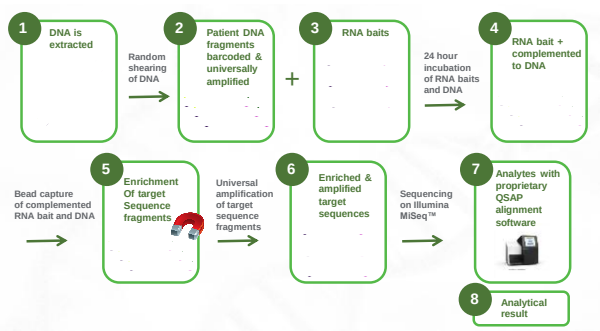


* Lifetime risk for cancer of any type, including breast

Genetic Testing Criteria and Management Guidelines

Gene	Genetic Testing Criteria	Management Guidelines
BRCA1/BRCA2	NCCN: Genetic/Familial High-Risk Assessment: Breast and Ovarian	
TP53		
PTEN		
CDH1	NCCN Clinical Practice Guidelines in Oncology: Gastric Cancer	
STK11	None (Clinical Diagnosis)	NCCN: Genetic/Familial High-Risk Assessment: Colorectal
PALB2	None	ACS recommends screening with MRI for women with at least 20% lifetime risk of breast cancer

BRCAvantage Plus™ Technology



Interpreting Results of Genetic Tests

- Interpretation Summary
- Color-Coded 5-Tier Classification
  - Known Pathogenic (**RED**)
  - Likely Pathogenic (**RED**)
  - VUS (**YELLOW**)
  - Likely Benign (not reported)
  - No Mutation Detected (**GREEN**)
- Comprehensive Interpretation
- ACMG Guidelines

Possible Test Results

POSITIVE (Pathogenic, Likely Pathogenic):

- Refer to NCCN Management Guidelines
- Relatives should consider single-site testing

NEGATIVE:

- Uninformative result versus true negative. True negative only if family history is due to a known mutation.
- Consider additional testing for your patient and/or other family members

VARIANT OF UNCERTAIN SIGNIFICANCE:

- Additional data required for interpretation (e.g., family studies, functional studies)
- Patient recommended to check in with MD/Quest Diagnostics on regular basis (e.g., every year) as variant status may change
- Management based on patient's personal and family history

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BRCA Share™

- Co-founded by Quest Diagnostics and Inserm
- Provides scientists and commercial laboratories around the world open access to *BRCA1* and *BRCA2* genetic data
- The goal of BRCA Share is to accelerate research on *BRCA* gene mutations and clarify the pathogenicity of variants of uncertain significance

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Noninvasive prenatal screening (NIPS)

QNatal™ Advanced

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- Overview
  - Quest Diagnostics
  - Chromosomal abnormalities
  - Evolution of prenatal screening
  - Clinical utility of NIPS
- Performance of QNatal Advanced
- Summary

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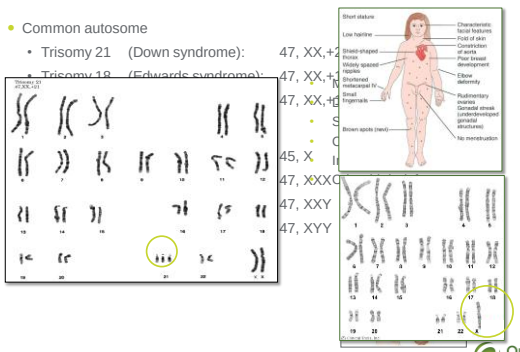
Chromosomal Abnormalities:
Aneuploidy and Microdeletions

Chromosomal Aneuploidy

- Common autosome
  - Trisomy 21 (Down syndrome): 47, XX,+21
  - Trisomy 18 (Edwards syndrome): 47, XX,+18
  - Trisomy 13 (Patau syndrome): 47, XX,+13
- Common sex chromosome
  - Monosomy X (Turner syndrome): 45, X
  - Triple X (Triple X syndrome): 47, XXX
  - Klinefelter (Klinefelter syndrome): 47, XXY
  - XYY (XYY syndrome): 47, XYY

Chromosomal Aneuploidy: Clinical Picture

- Common autosome
  - Trisomy 21 (Down syndrome): 47, XX,+2
  - Trisomy 18 (Edwards syndrome): 47, XX,+
  - 47, XX,+
  - S
  - C
  - 45, X I
  - 47, XXX
  - 47, XXY
  - 47, XYY



Chromosomal Microdeletions

- Microdeletion: a chromosomal deletion too small to be detected by standard cytogenetic analysis
- Microdeletion syndrome - Characteristics vary based on the size and location of the deletion
  - DiGeorge syndrome: 22q
  - 1p36 syndrome: 1p
  - Angelman syndrome: 15q
  - Prader-Willi syndrome: 15q
  - Cri-du-chat syndrome: 5p
  - Wolf-Hirschhorn syndrome: 4p
  - Jacobsen syndrome: 11q
  - Langer-Giedion syndrome: 8q

Chromosomal Microdeletions: Clinical Picture

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  - Wolf-Hirschhorn syndrome: 4p
  - Jacobsen syndrome: 11q
  - Langer-Giedion syndrome: 8q
    - Hyperphagia
    - Obesity
    - Intellectual Disability

National Institutes of Health. February 2014. http://ghr.nlm.nih.gov. Accessed February 11, 2015.

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Evolution of Screening:
MSS to NIPS

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Evolution of Fetal Aneuploidy Screening

1960s	1980s	1988	1996	1997	2011
Maternal Age	MSAFP	Triple Screen	Quad Screen	Integrated Screen + NT*	NIPS
Detection (Sensitivity) Rate (%)					
27	36	60 - 74	70 – 81	80 - 95	79 - >99**
Gestational Age (weeks)					
≥35 years at EDD	15 +	15 +	15 +	10 - 11 +	10 +
Chromosomal Abnormalities Detected					
All	T21	T21 T18	T21 T18	T21 T18	T21, T18, T13 **sex chromosome aneuploidy***, microdeletions*****

* Nuchal translucency
** Applies to T21, T18, and T13
*** Some cases

Devers PL. *J Genet Coun.* 2013;22(3):291-295

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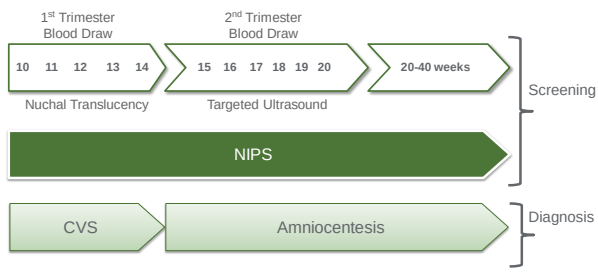
Current Options for Fetal Aneuploidy Screening



Driscoll DA. *Genet Med.* 2009;11(11):818-821.

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Clinical Utility of NIPS:
Action from Insight.

Clinical Impact of NIPS

Total abnormal amniocentesis results = 2.3%

Aneuploidy; Robertsonian translocation 1.86%

- T21 1.16%
- Sex chromosome aneuploidy 0.35%
- T18 0.23%
- T13 0.07%
- t(13:14) 0.05%

82% of total chromosomal abnormalities
Detectable now by NIPS*

97.7% of amniocenteses have normal results

Marker; unbalanced translocation 0.12%
Other 0.09%

9% of total chromosomal abnormalities
Reported detectable in literature by MPSS**

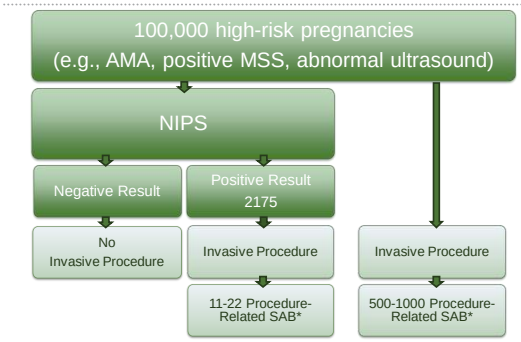
Balanced arrangements 0.20%

9% of total chromosomal abnormalities
Unlikely detectable by MPSS**

*NIPS: Noninvasive prenatal screening
**MPSS: Massively parallel shotgun sequencing.

Ferguson-Smith MA. Prenat Diagn. 1984 Spring;4 Spec No:5-44.

Clinical Impact of NIPS



*Based on spontaneous abortion (SAB) rate of 0.5-1%

McCullough et al. *PloS One*, 2014

Clinical Impact of NIPS on Invasive Prenatal Diagnosis

- Action from insight
  - Provides invaluable insight for informed decisions with regard to electing CVS or amniocentesis.
- Can be used as 1st tier screening (high-risk pregnancy) or 2nd tier screening (general and high-risk pregnancy)
- QNatal Advanced detection (sensitivity) rate = 100%
  - Validation series, confidence interval = 89.95% - 100%
  - General NIPS sensitivity rates range from 70% to 94%
    - Includes sex chromosome aneuploidy and microdeletions
- Higher positive predictive value (PPV) than MSS, NT, or a combination of both
- NIPS is screening; it is not diagnostic
  - False positives and negatives do occur; therefore, an invasive test is required to definitively confirm any positive result.

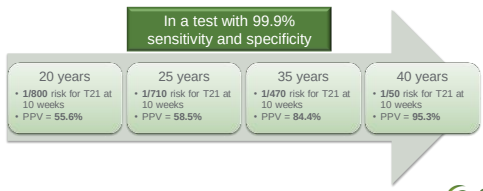
Benn PA. Obstet Gynecol. 2002;100(6):1168-1176.; Malone FD. N Eng J Med. 2005;353:2001-2011.; Swanson A. Curr Genet Med Rep. 2013;1(2):113-121.; Wang JC. Genet Med. 2014. doi: 10.1038/gim.2014.92.

Clinical Complications of NIPS: False Positives and Negatives

- Confined placental mosaicism (CPM)
- Co-twin demise/vanishing twin
- Fetal mosaicism
- Maternal mosaicism
- Maternal malignancy
- Collection/laboratory error
- Directly related to prevalence (positive and negative predictive value)

Positive and Negative Predictive Value (PPV and NPV)

- Unlike sensitivity and specificity, PPV and NPV are dependent upon condition prevalence
  - PPV: percentage of patients with a positive test who truly have the condition
  - PPV (%) = $\frac{\text{(true positive)}}{\text{(true positive + false positive)}} \times 100$
- PPV for NIPS is lower in the general pregnancy population, as the incidence of chromosomal aneuploidy is lower in these patients:



Wang JC. *Genet Med*. 2014. doi: 10.1038/gim.2014.92.

Summary of ACOG, ISPD, and NSGC Recommendations

- NIPS should be included in prenatal testing options offered to high-risk patient population
- NIPS should be offered within the context of informed consent, education, and counseling by a qualified provider
- NIPS positive results should be followed by recommending an option for confirmatory diagnostic testing
  - Negative results do not ensure an unaffected fetus

American College of Obstetricians and Gynecologists Committee on Genetics. Obstet Gynecol. 2012;120:1532-1534.
Devers PL. J Genet Coun. 2013;22(3):291-295.
Benn P. Prenat Diagn. 2012;32(1):1-2.

QNatal Advanced

Performance Snapshot

QNatal Advanced Test Content

Location	Syndrome Name	Estimated Frequency of Live Birth
Chromosome 21	Down	1/800
Chromosome 18	Edwards	1/6,000
Chromosome 13	Patau	1/7,000-33,000
Chromosome X	X, XXX, XXY, XYY	1/500
Sex Chromosomes	Sex Determination	
22q*	DiGeorge	1/4,000
1p*	1p36	1/10,000
15q*	Angelman	1/20,000
15q*	Prader-Willi	1/20,000
5p*	Cri-du-chat	1/50,000
4p*	Wolf-Hirschhorn	1/50,000
11q*	Jacobsen	1/100,000
8q*	Langer-Giedion	Rare

*Microdeletion

National Institutes of Health. February 2014. http://ghr.nlm.nih.gov. Accessed February 11, 2015.

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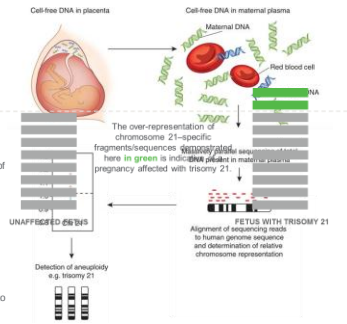
Performance of QNatal Advanced

- NIPS detects cell-free fetal DNA (cfDNA) of trophoblastic origin from the plasma of pregnant women
  - ~10% of DNA fragments in a pregnant woman's plasma are fetal
- MPSS platform is used to detect maternal and fetal cfDNA
  - MPSS ***can*** elucidate the chromosome of origin for a particular DNA fragment (see next slide)
  - MPSS ***cannot*** differentiate between maternal and fetal DNA fragments
- Bioinformatic analysis is used to determine the representation of fetal cfDNA
  - **GC normalization**: routine correction to account for suboptimal amplification of GC-rich regions



Bianchi DW. *Nat Med*. 2012;18(7):1041-1051.; Bianchi DW et al. *Obstet Gynecol*. 2012;119(5):890-901.; Benn P. *Prenat Diagn*. 2012;32(1):1-2.; Zhao C et al. *PloS One*. In press

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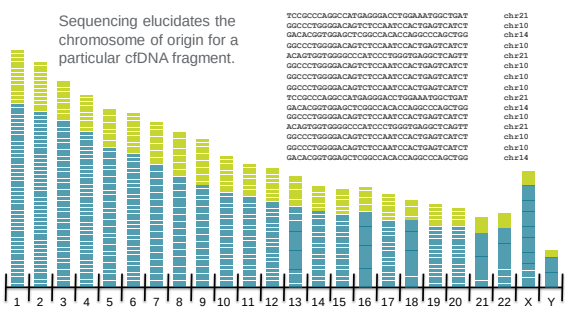
Performance of QNatal Advanced

Sequencing elucidates the chromosome of origin for a particular cfDNA fragment.



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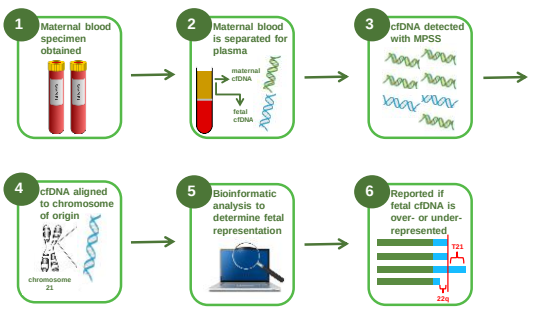
Performance of QNatal Advanced



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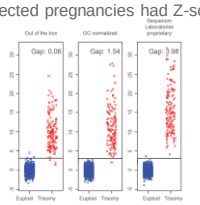
Validation of QNatal Advanced: Z-score Calculation

- Z-Score is used to determine positive/negative result for core trisomies
  - i.e., over-representation of chromosome material vs. appropriate representation of chromosome material
  - Z-Score: number of standard deviations from the mean (average)
  - Z-Score (chromsome 21) = (chromosome 21% - mean chromosome 21%) / (standard deviation chromosome 21%)
- In assay development, all trisomies had Z-scores greater than 11 when fetal fraction was greater than 5%
- In validation, all unaffected pregnancies had Z-scores less than 4



Zhao C et al. *PloS One*. In press

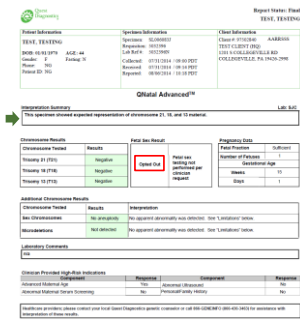
Summary of QNatal Advanced

- Our laboratory-developed NIPS
- Can be performed as early as 10 weeks' gestation
  - At any gestational age thereafter without restriction
- Whole genome massively parallel shotgun sequencing (MPSS)
- Measures and reports fetal fraction as either sufficient or insufficient for analysis using a 5% cut-off
- Binary positive/negative result for core trisomies (T21, T18, T13)
- Clear reporting of sex chromosome aneuploidy and microdeletions as additional, incidental findings
- Opt out selections for microdeletions and fetal sex
- Validated for use in twin gestation
- 1-2% "no call" rate based on internal validation data

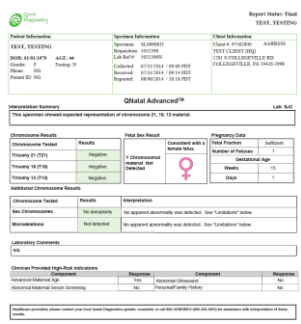
QNatal Advanced
Sample Reports

QNatal Advanced Sample Report: Clear and Concise

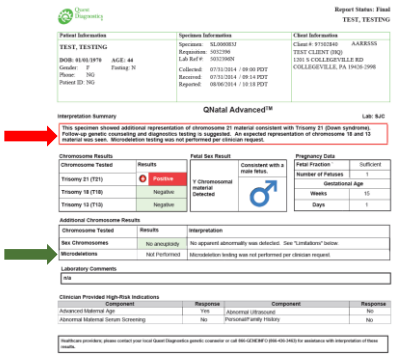
Negative, Fetal Sex Opted Out



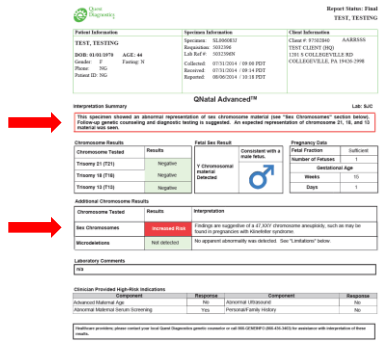
Negative, Female



QNatal Advanced Sample Report: Positive T21, Microdeletions Opted Out

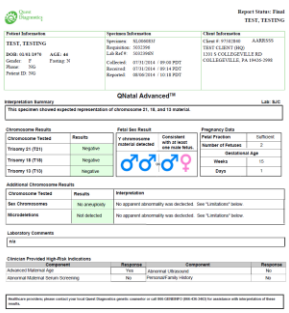


QNatal Advanced Sample Report: XXY Male Finding

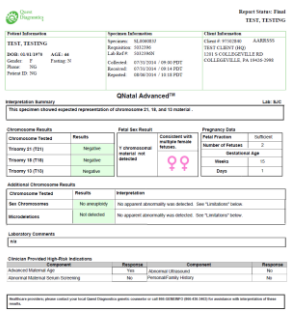


QNatal Advanced Sample Report: Clear and Concise

Negative, Male Twin(s)



Negative, Female Twins



Thank You

QUESTIONS?