An overview of prenatal cf-DNA screening methods; clinical efficacy and scope.

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Interest statement: Consultant to Natera, Inc.

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In this presentation:

- Review performance for the most widely used NIPT methods
- Summarize the main technical differences in the approaches to cf-DNA screening
- Scope of testing and other differences in available tests
  - Sex chromosome abnormalities
  - Microdeletions
  - Triploidy
  - Genome-wide (separate talk)
  - Twins
  - Low fetal fraction
  - Single gene disorders (separate talk)

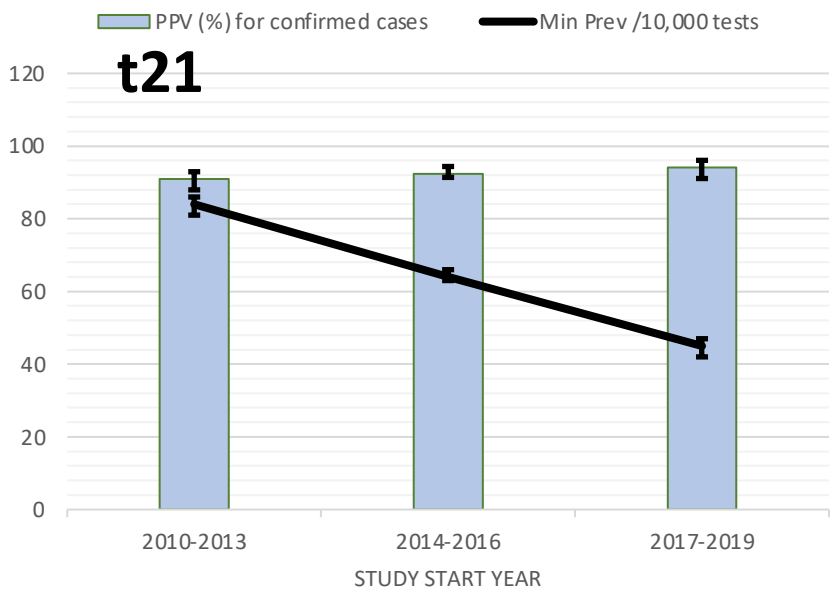
Performance of cf-DNA screening: meta-analysis

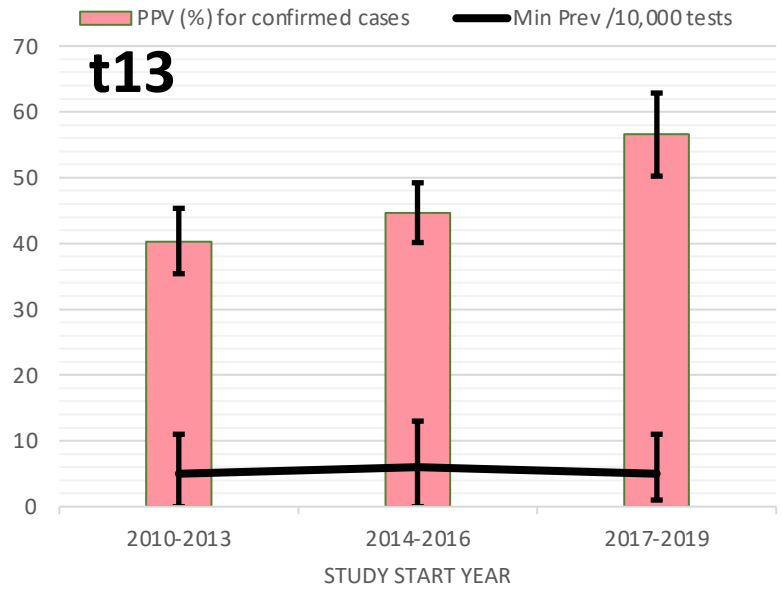
	Studies	No. called	DR [95%CI] (%)	FPR [95%CI] (%)
Trisomy 21				
Retrospective	16	11,847	99.1 [98.2-99.6] (a)	0.21 [0.08-0.57]
Prospective	24	57,892	98.0 [96.4-98.9] (a)	0.09 [0.05-0.17]
Clinical	75	2,423,541	-	0.05 [0.05-0.05]
Trisomy 18				
Retrospective	13	11,181	96.5 [92.3-98.4]	0.17 [0.08-0.35]
Prospective	23	57,049	92.8 [88.7-95.5]	0.09 [0.05-0.18]
Clinical	73	2,412,830	-	0.04 [0.04-0.04]
Trisomy 13				
Retrospective	12	10,737	88.4 [76.6-94.6]	0.10 [0.03-0.38]
Prospective	21	49,321	93.2 [84.5-97.2]	0.08 [0.04-0.14]
Clinical	73	2,401,602		0.05 [0.05-0.05]
Monosomy X				
Retrospective	9	5,499	93.8 [86.1-97.4]	0.45 [0.09-2.13]
Prospective	8	6,880	76.1 [49.1-91.3]	0.33 [0.20-0.54]
Clinical	42	2,094,493	-	0.10 [0.09-0.10]

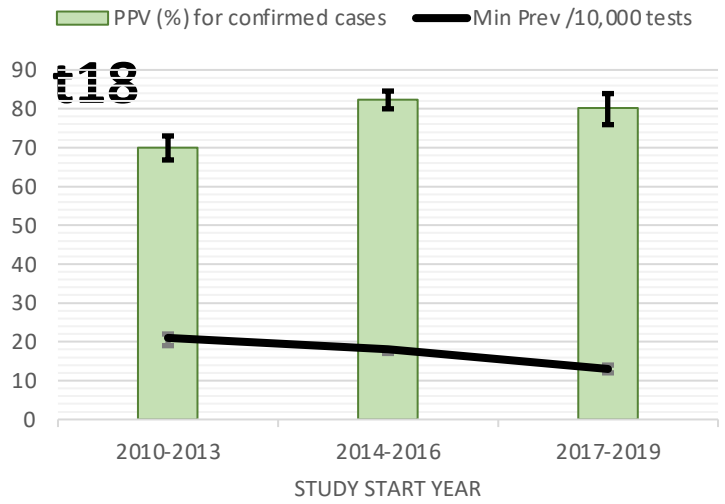
(a) Prospective and retrospective trial data based on bivariate random effects model. (b) Clinical data based on pooled data

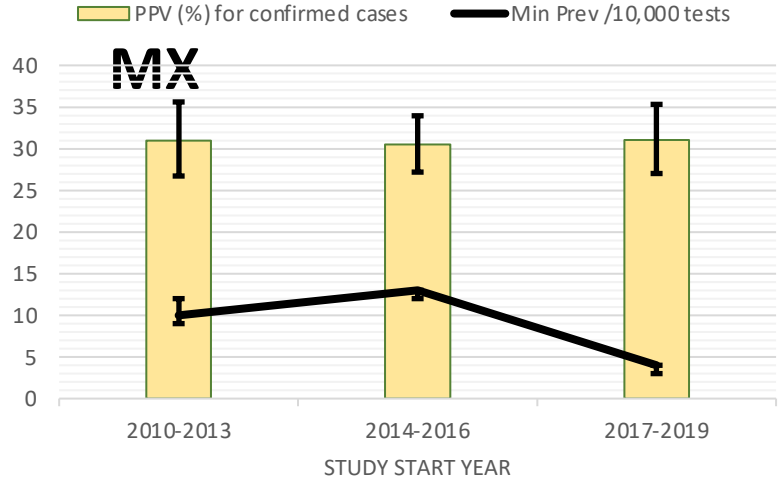
Demko, Prigmore & Benn. J Clin Med. 2022;11:4760.

Trends in clinical experience papers: testing in lower risk patients but steady PPV







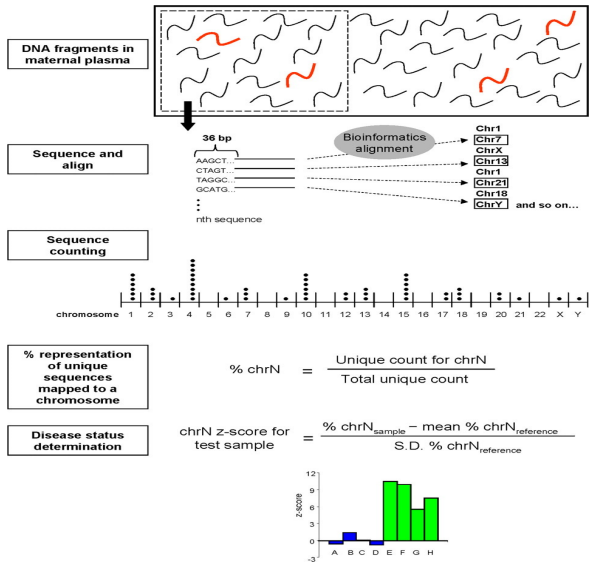


Black line: Minimum prevalence (based TP and known FN)

Colored bars: PPV

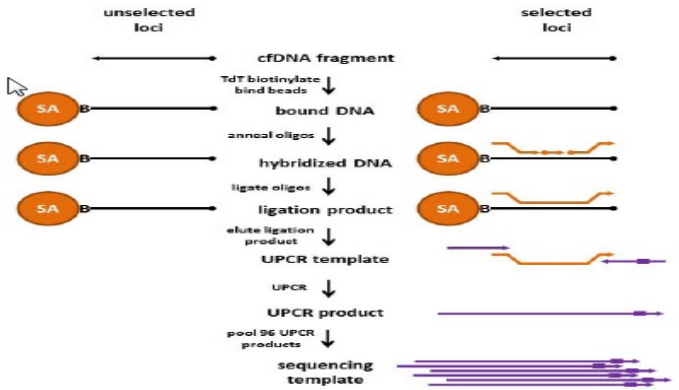
Based on Demko, Prigmore & Benn. J Clin Med. 2022;11:4760.

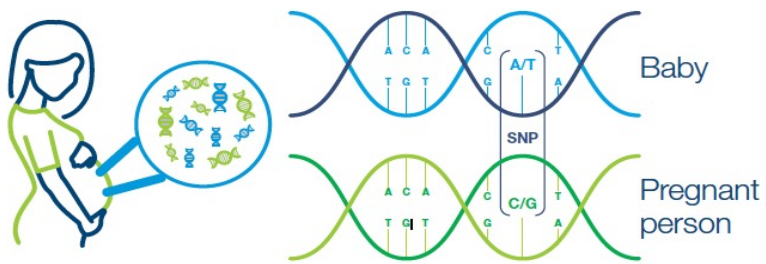
The most widely used cf-DNA screening methods



massively parallel shotgun sequencing (MPSS)

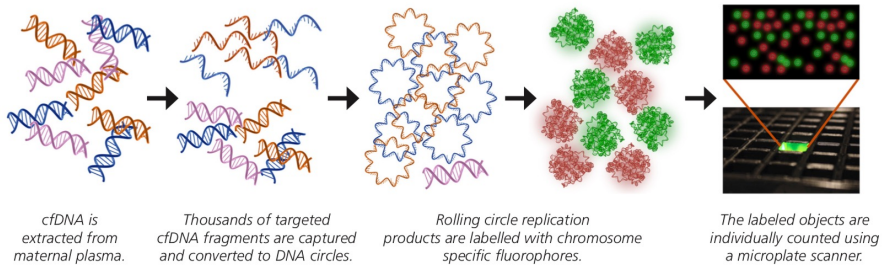
Digital analysis of selected regions (DANSR)





SNP-based analysis

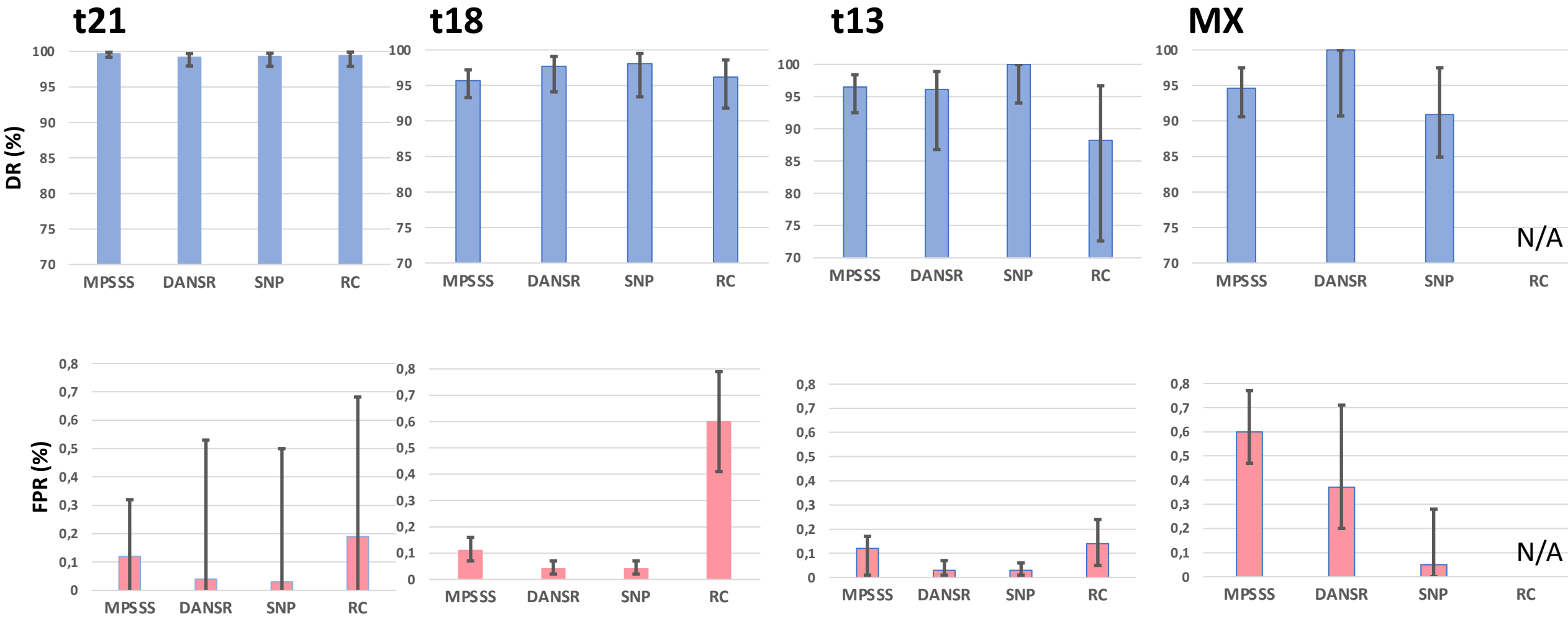
Rolling circle



What do these methods have in common; how do they differ?

Same	Different
Cf-DNA at 9+ or 10+ weeks gestational age	
	Use of enrichment or amplification of chromosome regions
	Use of enrichment of fetal DNA
	Use of sequencing, array, or fluorescent signals for detection
	Analytic computations, cut-offs
	Performance at low fetal fraction
	Chromosome regions analyzed, principal component analysis
	Validation study design, refinements
	Cost

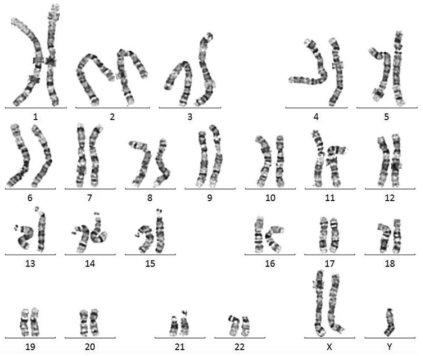
Performance: t21, t18 and t13:by method, all validation studies, aggregate data



Demko, Prigmore & Benn. J Clin Med. 2022;11:4760.; Benn and Cuckle. Clin Obstet Gynecol. 2023. 66; 536-556.

Other sex chromosome abnormalities

		MPSS	DANSR	SNP-based	RC
Sex chromosome abnormalities		Yes	Yes	Yes	No



Performance of cf-DNA screening: SCA meta-analysis

	Studies	No. called	DR [95%CI] (%)	FPR [95%CI] (%)
47,XXY	16	11,248	100 (99.6-100)	0.01 (0-0.06)
47,XXX	13	10,255	100 (96.9-100)	0.11 (0.01-0.29)
47,XYY	9	8,473	100 (91.3-100)	0 (0-0.02)

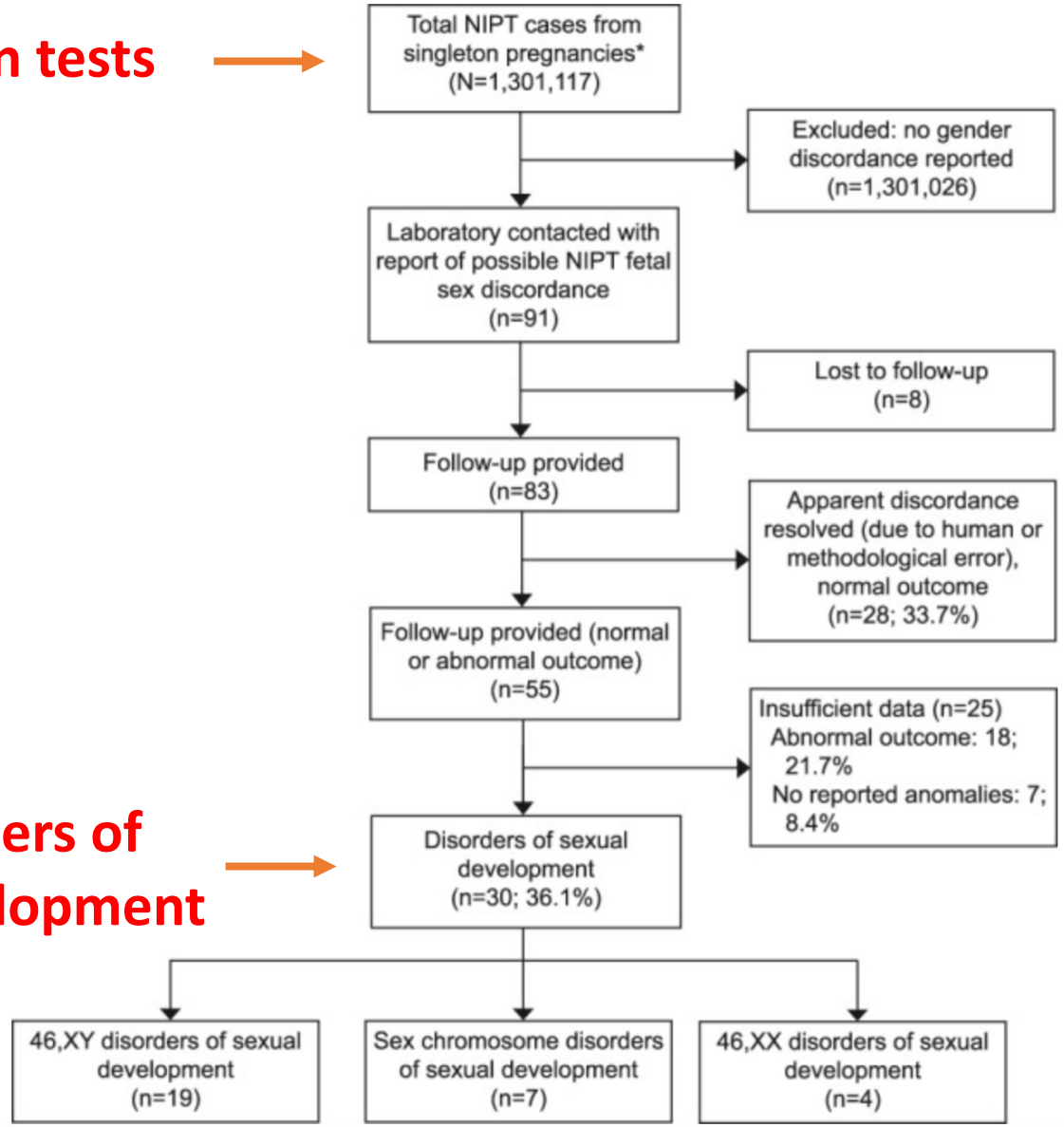
Caveats:

1. FPs may be under-ascertained due to low numbers of confirmatory tests
2. FNs will not come to attention.

Shear et al., Prenat Diagn 2023;43(2):133-143

Fetal sex determination; consistency with ultrasound

1.3 million tests



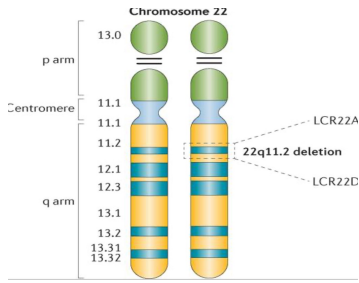
28 errors

30 Disorders of sexual development

Dhamankar et al., Obstet Gynecol. 2020; 135:1198-1206.

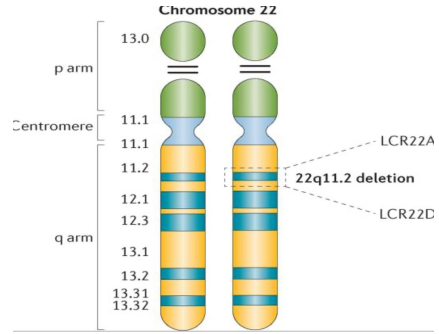
Select Group of Microdeletions

		MPSS	DANSR	SNP-based	RC
22q11.2 DS*		Some labs	Yes	Yes	No
Select other microdeletions		Some labs	Yes	Yes	No
All microdeletions and duplications detectable by CMA		No	No	No	No



22q11.2 deletion syndrome (DiGeorge/VCFS)

- Prenatal incidence ~1 in 1,000 - 1:2000
- Deletions are ≤ 3 Mb, less than 1/10 the size of chromosome 21
- Many cases are not diagnosed at birth (few prenatally, some not until adulthood)
  - 75% with congenital heart defects
  - 75% with immune deficiencies
  - 30% with feeding difficulties requiring feeding tube
  - 35% with malformed or missing kidney
  - 10% born with cleft palate
  - Variable developmental delay and learning disabilities
  - 25% develop schizophrenia in young adulthood

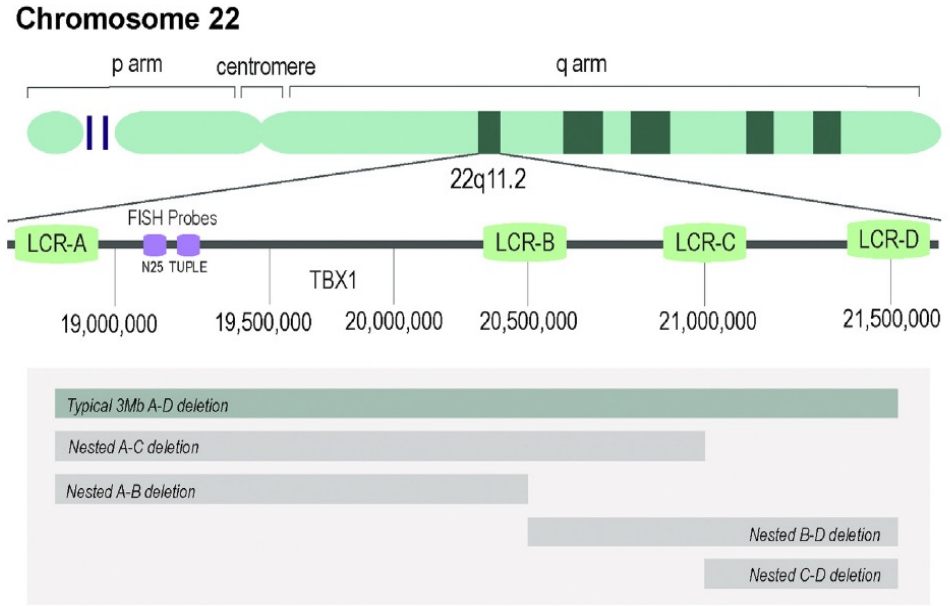


Early detection can potentially:

- Reduce seizures and thereby reduce long-term cognitive impairment
- Result in deliveries at tertiary healthcare centers where cardiac and other malformations can be managed
- Avoid “diagnostic odyssey”
- Reduce healthcare costs

The “SMART” prospective cf-DNA screening study for 22q11.2 deletion syndrome

- 20,887 singleton pregnancies with outcome known for 18,289 (87.6%)
- Deletion (including nested) evaluated by SNP-based NIPT.
- DR= 9/12 (75%); 3/5 nested deletions detected
- FPR= 29/18,002 (0.16%)
- PPV= 23.7%
- Prenatal prevalence 1 in 1,524
- Revised protocol proposed with PPV increased to 52.6%

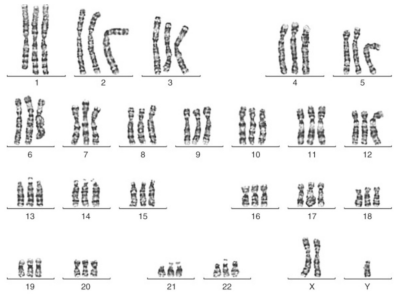


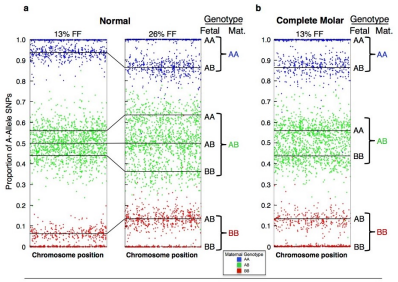
Not generalizable to other methodologies, other microdels and microdups
A panel of CNVs will have low PPVs (due to rarity) and a cumulative FPR

Dar et al., 2022. AJOG. Am J Obstet Gynecol. 2022;227:79.

Triploidy

		MPSS	DANSR	SNP-based	RC
Triploidy/ Complete moles		No	No	Yes	No





Triploidy and complete molar pregnancies

- Estimated incidence of triploidy: ~1 in 4,800 pregnancies at 11-13 weeks
- Digynic cases are associated with growth retardation and fetal abnormalities
- Diandric cases show fetal abnormalities, partial molar placentas, and a risk for gestational trophoblastic disease (GTD)
- Identifiable through the presence of an extra haplotype by SNP analysis
- Results can also be attributable to vanished twin, undetected twin, and various more complex explanations.

Kantor et al., 2022 Prenat Diagn 42:994-9.

Twins, higher multiples

		MPSS	DANSR	SNP-based	RC
Twins, autosomal chromosome abnormalities		Yes	Yes	Yes	Yes
Twins, sex chromosome abnormalities		Some labs	No	No	No
Higher multiples		Some labs	?	No	?

NIPT in twin pregnancies

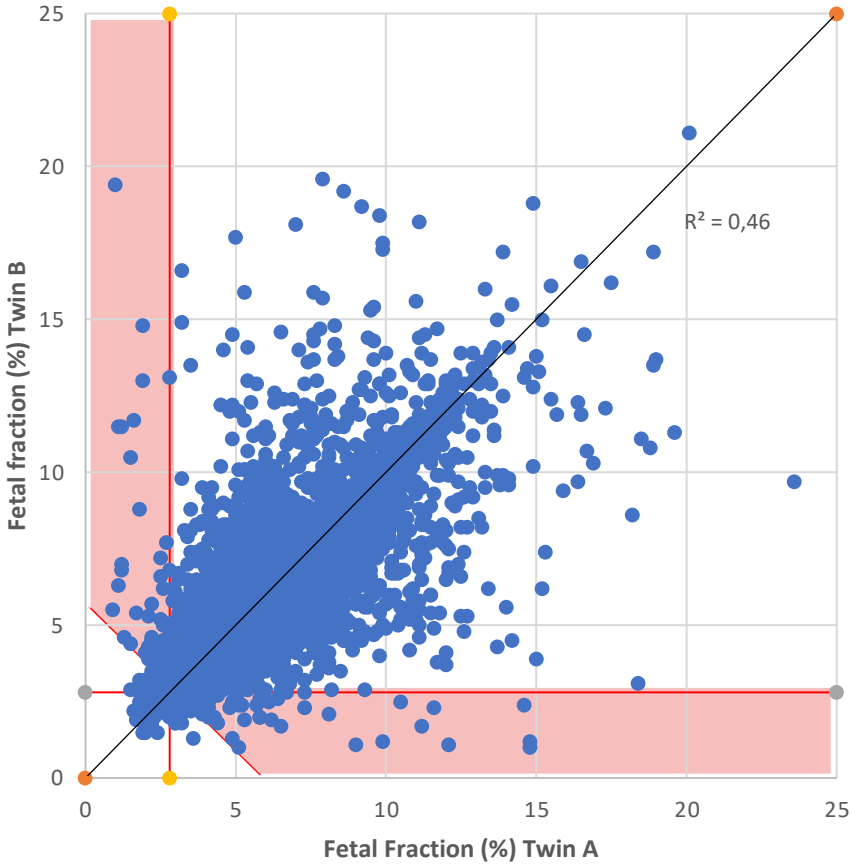
- All major approaches provide NIPT results in twin pregnancies
- Overall performance is less than for singletons; but far superior to conventional serum markers
- For monozygotic twins, testing performance should be better than singletons
- For dizygotic twins, the two FFs can vary widely; aggregate FF is not an adequate measure
- SNP-based NIPT can provide information on zygosity[1]
  - (a) useful when chorionicity was not definitively established, detecting dizygosity substantially excludes monochorionicity
  - (b) and useful when one, or both, fetuses have abnormalities

Benn P, Rebarber A. Prenat Diagn. 2021;

Use of SNPs to measure individual fetal fractions in twin pregnancies

	Number of cases	Fetal Fraction (%) Mean ± sd	% cases with FF<2.8% (no call)
Singleton	136,667	9.7 ± 4.4	1.7%
Monozygotic (combined)	1,624	12.8 ± 5.1	0.7%
Dizygotic (combined)	3,521	13.0 ± 4.9	5.9% (one or both)
Dizygotic (per fetus)	7,042	6.5 ± 2.7	

Scatterplot of paired FFs in DZ pregnancies

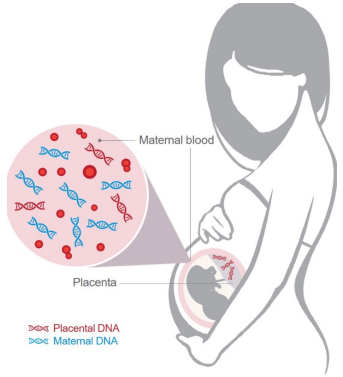


Hedriana, H et al. Prenat Diagn. 2020 Jan;40(2):179-184.

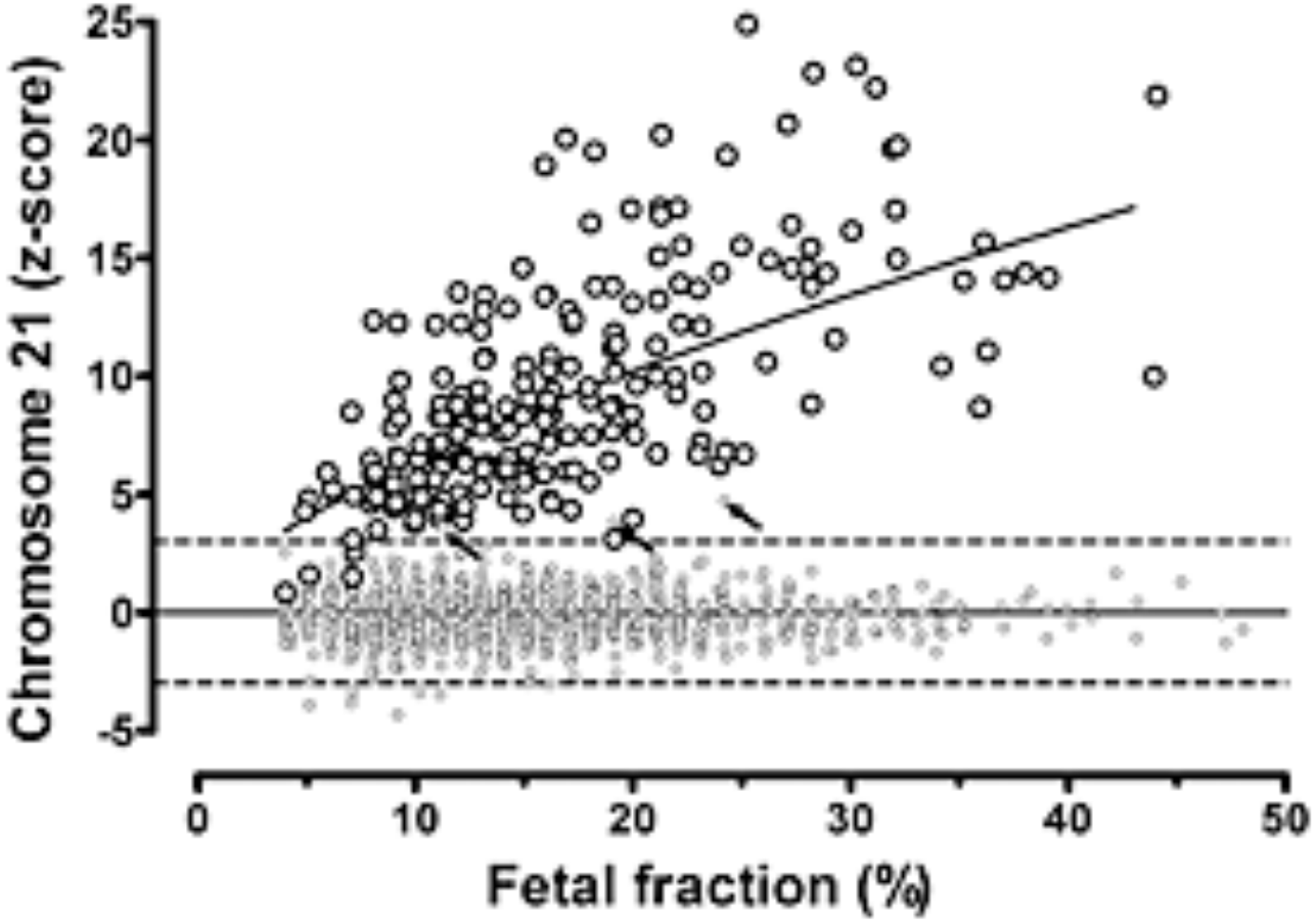
Low fetal fraction

		MPSS	DANSR	SNP-based	RC
Measures and reports FF*		Some labs	Yes	Yes	No

* Different criteria are used to define the cut-off for “low”” FF



Is FF measurement necessary?



Canick et al., 2013; Prenat Diagn. 33:667-74.

Is it important to measure fetal fraction?

Measuring FF is unimportant because:	Measuring FF is important because:
Only a small proportion of cases have low FF and therefore it makes very little difference in the detection of DS	The low FF is enriched for t18, t13, digynic triploidy, and perhaps other abnormalities.
FF cannot be measured accurately and wrongly assigned values could diminish DR	We need confidence in results, particularly for patients who are obese, have autoimmune disease or other conditions that affect FF.
	FF can be expected to be particularly crucial in detecting microdeletions
	Low FF can be an indicator for loss, other adverse outcomes
	Provides an explanation for some discordances, e.g., fetal sex

“Low” FF and the risk of chromosome abnormality: a meta-analysis

Abnormality	Odds ratio (95% CI)	Significance	Placenta size
t21	1.25 (0.76-2.03)	Non-Significant	Normal
t18	4.46 (3.07-6.47)	Significant	Small
t13	5.99 (3.61-9.95)	Significant	Small
MX	5.88 (2.34-14.78)	Significant	Normal or hydropic
Triploidy	36.39 (9.83-134.68)	Significant	Small placenta for digynic
Other”	4.00 (1.78-9.00)	Significant ??	Depends on abnormality

Cannot rule out ascertainment bias; cases with low FF or small placenta may be more likely to be referred for diagnostic testing

Becking et a., lPrenat Diagn. 2023;43(7):838-853

Genome-wide large abnormalities (~>7Mb)

Single gene tests

To be discussed elsewhere in this conference

Summary

- NIPT for the common trisomies is highly effective although there are differences between assays
- Testing appears to have improved with time and experience
- Different methods have strengths and weaknesses with respect to the detection of other cytogenetic abnormalities
- Careful attention needs to be paid to QA aspects which includes fetal fraction and the accuracy of fetal sex determination

Scope: Other cytogenetic abnormalities detected by different methods

		MPSS	DANSR	SNP-based	RC
Sex chromosome abnormalities		Yes	Yes	Yes	No
22q11.2 DS*		Some labs	Yes	Yes	No
Select other microdeletions		Some labs	Yes	Yes	No
All microdeletions and duplications detectable by CMA		No	No	No	No
Triploidy/ Complete moles		No	No	Yes	No
Genome-wide imbalances >7Mb		Some labs	No	No	No

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Prenatal prevalence is ~ 1 in 1500 for typical 3Mb plus nested deletions. PPV> 50% in one study. (Dar etal', AJOG 2022.
Deletions are 1/10 the size of a chromosome 21.

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