Supplement 5 Table of study characteristics of included studies

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Alberti 2015[56] France Study start date: March 2010	Prospective case-control (cases with abnormal karyotype matched with a balanced number of randomly selected pregnancies with euploid karyotypes) Number of centres: 3	N=976 enrolled in cohort. Women with singleton pregnancies, high-risk of foetal T21. N=225 in case-control for sequencing. Mean age (SD): 35.2 (6.7) years. Mean gestational age (SD): 14 (2) weeks. 1st and 2nd trimester.	N=0 from cohort. N=751 (76.9%): Not included in case-control study.	T21	All high risk for foetal T21 (>1:250) based on the combination of maternal age with ultrasound and maternal serum markers during the first or second trimester.	MPS (whole genome) performed in a cytogenetics laboratory in a university teaching hospital	CVS or amniocentesis and foetal karyotype	None	NIPT performance for T21 detection.	Accuracy of NIPT
Ashoor 2012[46] UK	Nested case-control of stored maternal	N=400 (50 T21, 50 T18, 300 euploid)	Pregnant by IVF or multiple pregnancy	T21, T18	All high risk: Combined 1st trimester screen	DANSR, FORTE	Karyotyping after CVS	None	FORTE risk score for aneuploidies, sensitivity and specificity for	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Study start date: NR	samples: Controls matched with T21/T18 cases for sample storage time in 3:1 ratio. Number of centres: 1	Singleton pregnancies, high-risk women. 1st trimester 100%; All 11-13 weeks' gestation. Ethnicity: White 89%, 'Afro Caribbean' 5%, South/ East Asian 6%, Mixed 0.5%.	N=NR		risk >1:300	Aria Diagnostics (USA)			detection of T21 and T18	
Beamon 2014[36] USA Study start	Prospective cohort Number of centres: 1	N=208 High-risk pregnancies who chose NIPT as triage test, singleton or dichorionic twin gestations, ≥10	Multiple pregnancy N=NR	T21, T18, T13	All high-risk: AMA: 148 (71.2%), AMA alone: 121 (58.2%),	MPS (whole genome) Sequenom Center for	Karyotyping after amniocentesis, cordocentesis or	None	Test performance for T13, T18 and T21 detection.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
date: January 2012		weeks' gestation. Mean age (SD), range: 36 (5.5), 19-47 years. Mean gestational age (SD), range: 15.6 (4.3), 10-34 weeks. Trimester: 1st: 111 (53.4%), 2nd: 95 (45.7%), 3rd: 2 (1%).			AMA + other: 27 (13.0%), Ultrasound abnormality: 26 (12.5%), Abnormal serum screen: 29 (13.9%), Combined FTS: 16 (7.7%), Quadruple: 12 (5.8%), Integrated: 1 (0.5%), Affected family member: 3 (1.4%), Other: 2 (1.0%), Twins (growth discordance): 1 (0.5%), Maternal anxiety:	Molecular Medicine (USA) (n=163, 78.4%) or Verinata Health (USA) (n=45, 21.6%).	CVS, phenotype of newborn			

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
					1 (0.5%).					
Bevilacqua 2015[37] Belgium, UK, Spain Study start date: May 2013	Prospective multicentre cohort Number of centres: NR	N=515 included. Twin pregnancies at mixed risk for aneuploidies. Median gestational age (range): 13.0 (10.0-28.0) weeks. 1st trimester: 68.5%.	Criteria for exclusion from study NR	T21, T18, T13	Mixed risk: High risk for foetal trisomy by 1st-trimester combined test or 2nd-trimester triple/quadruple test or ultrasound or NIPT as primary method of screening.	DANSR, FORTE Harmony Prenatal test Ariosa Diagnostics (USA)	Karyotyping after amniocentesis, cordocentesis or CVS, or newborn phenotypic examination	None	1) Factors influencing failure rate in twin and singleton pregnancies. 2) NIPT performance for T13, T18 and T21 detection in twins.	Accuracy of NIPT
Bianchi 2012[47] USA Study start date: NR	Nested case-control Controls un matched in 4:1 ratio (Part of MELISSA	N=2,882 in cohort. N=534 in nested case-control study. Singleton pregnancies, high risk.	257/2,882 (8.9%) from MELISSA cohort: 85 multiple pregnancies, 45 no karyotype information, 127 ineligible	T21, T18, T13	All high risk: AMA (>38 years) only 152 (28.5%); Positive screen risk 91 (17.0%); Ultrasound abnormality	MPS (whole genome) Verinata-Illumina (USA)	Karyotyping after CVS	None	1) MPS performance (sensitivity and specificity) for T21, T18 and T13 detection.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
	prospective cohort). Number of centres: 53 (of 60)	Mean age (SD), range: 35.2 (6.40), 18 – 46 years. Mean gestational age (SD), range: 15.1 (3.16), 10 – 23 weeks. Trimester: 1st: 165 (30.9%), 2nd: 369 (69.1%). Ethnicity: White 72.7%, African American 10.9%, Asian 9.9%, Native American or	blood sample.		122 (22.8%); Prior aneuploidy pregnancy 15 (2.8%); More than 1 risk 154 (28.9%).				2) Sex chromosome classification and Monosomy X detection.	

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Alaska Native 0.9%, Multiracial 5.6%.								
Bianchi 2014[19] USA Study start date: July 2012	Prospective cohort Number of centres: 21	N=2,052 enrolled. N=2,042 eligible. Singleton pregnancies, general obstetric population. Trimester: 1st: 759 (39.7%), 2nd: 610 (31.9%), 3rd: 545 (28.5%). Mean gestational age (SD), range: 20.3 (8.6), 8.0 – 39.4 weeks.	N=10 (0.5%): 7 insufficient blood volume, 1 late receipt of blood sample, 1 maternal age <18 years, 1 withdrawn consent.	T21, T18, T13	General obstetric population undergoing standard prenatal aneuploidy screening	MPS (whole genome) Verifi Verinata-Illumina (USA)	Newborn phenotype (97.0%) or Karyotyping (3.0%).	Standard prenatal aneuploidy screening produced by accredited clinical laboratories. Cutoff values as used by individual laboratories 1st-trimester: Combined test (PAPP-A, β-hCG, NT) N=739 (38.6%).	1) Comparison of false positive rates of NIPT with conventional screening for T21 and T18. 2) Comparison of false positive rates for T13. Comparison of foetal fractions in low-risk with high-risk patients.	Comparison of NIPT with CT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Mean age (SD), range: 29.6 (5.54), 18.0 – 48.6 years. Assisted conception 66 (3.4%).						2nd-trimester: Quadruple (MS-AFP, β-hCG, estriol and inhibin A) N=439 (22.9%); Quadruple + combined test N= 53 (2.8%); Quadruple + 1st-trimester serum markers only N=164 (8.6%); Sequential: 1st-trimester screen results reported before final report in 2nd trimester N=519 (27.1%).		
Chen 2011[48] Hong Kong,	Case-control of stored samples and	N=392 (N=140 archived plasma samples with	NR	T18, T13	All high risk based on clinical indicators as per the existing	MPS (whole genome)	Karyotyping after CVS or amniocent	None	Diagnostic performance of MPS for T13 and T18	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
UK, Netherlands, China Study start date: NR	prospectively recruited women Number of centres: 10	and without aneuploidy matched for gestational age; N=252 prospectively recruited.) 344/392 samples analysed in a previous study [49], 48 cases newly recruited. Singleton pregnancy undergoing CVS/amniocentesis.			obstetric practice of each recruitment unit.	Sequenom (USA)	esis		detection.	
Chiu 2011[49] Hong Kong, UK, Netherlands, China Study start date: October	Case-control of stored samples and prospectively recruited women Number of	N=824 screened (N=248 archived T21 and non-T21 samples matched for gestational ages in 1:5 ratio and N=576 prospectively collected high-risk	N=60 (7.3%): 14 failed recruitment criteria (2 twin pregnancies, 12 without full	T21	High risk by conventional screening (>1:300): 582 (77%), Median risk for T21: 1 in 43.	MPS (whole genome) Sequenom (USA)	Full karyotyping after amniocentesis (18%) or CVS (82%).	None	Diagnostic sensitivity, specificity, PPV & NPV for T21 detection.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
2008	centres: 10	samples), N=764 included. Singleton pregnancies. Median age: 35.4 years. Median gestational age: 13+1 weeks. 1st trimester: 74%.	karyotyping); 46 compromised blood sample (3 samples collected after invasive obstetric procedure, 2 delayed blood processing, 3 with ambiguous information, 12 haemolysed, 26 inadequate volume).		Intermediate risk by conventional screening (1:300-1:1000) 39 (5%), Median risk for T21: 1 in 502. Other indications (previous T21 pregnancy, ultrasound abnormalities, risk for monogenic diseases).					
Comas 2014[38] Spain Study start date: January	Prospective cohort Number of centres: 1	N=333 Singleton pregnancies who chose to have NIPT. Mean maternal age	Multiple pregnancies, ultrasound anomalies or high risk of congenital malformation N=NR	T21, T18, T13	Routine general population in a real clinical setting. 83.5% Low-risk by conventional	DANSR FORTE (Harmony Prenatal Test), Ariosa Diagnostics	Invasive testing and karyotyping or newborn phenotype.	None	1) NIPT test performance for T13, T18, and T21. 2) Comparison	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
2013		(range): 37 (21-46) years. Mean gestational age (range): 14.6 (9.5-23.5) weeks. 1st and 2nd trimester.			screenings but unable to alleviate their anxiety. 16.5% High-risk from CT or referred for AMA with no prior screening.	(USA) (n=120, 36.0%) or SNP- and NATUS (Panorama) Natera Inc. (USA) (n=213, 64.0%)			of Harmony and Panorama tests, factors influencing foetal fraction.	
Dan 2012[63] China Study start date: 1st quarter 2010	Prospective multicentre cohort Number of centres: 49	N=11,263 recruited. N=11,184 included. Singleton pregnancies, ≥ 18 years, gestational age of 9 - 28 weeks. Median age (range): 31 (18-49) years.	N=79 (0.7%): 55 unqualified gestational age, 14 multiple pregnancies, 10 foetal death.	T21, T18	Mixed risk factors Conventional T21 screening test: yes - positive: 4,522 (40.7%) yes - negative: 2,426 (21.8%) No – with 1 or	MPS (whole genome) BGI-Shenzen (China)	Full karyotyping 3,000 (26.6%) or birth questionnaire 4,524 (40.2%).	None	1) Sensitivity and specificity of MPS for T21 and T18 screening. 2) Workflow of MPS-based test.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Median gestational age (range): 20 (9-28) weeks. 2nd trimester: >74%. 42/49 centres offered test to high-risk pregnant women identified by a conventional T21 screening test, 7/49 centres enrolled participants regardless of prior risk assessment.			more other risk factors (≥ 35 years, family history of aneuploidies, ultrasound abnormalities): 2,770 (24.9%) No – without any risk factors: 1,387 (12.5%).					
Del Mar Gil 2014[21] UK Study start	Retrospective cohort of stored samples Number of	N=207 Twin pregnancies undergoing first-trimester screening for trisomies by combined test.	Singleton pregnancies N=NR	T21, T18, T13	NR	DANSR FORTE Harmony	Known birth outcome	None	Performance of Harmony Test in twin pregnancies only	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
date: NR	centres: 1	Age range: 26 – 41 years. Gestational age, range: 11 - 13 weeks. 1st trimester: 100%. Ethnicity: Caucasian 70.0%, Afro-Caribbean 23.7%, South/East Asian 1.0%, Mixed 5.3%.				Ariosa Diagnostics (USA)				
Dhallan 2007[57] USA	Prospective observational study	N=60 Women ≥ 18 years, singleton pregnancy.	N=NR	T21	Mostly high risk. Definition unspecified.	SNP allelic ratio	Amniocentesis or newborn reports	None	Performance of SNP method in detecting T21	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Study start date: January 2004	Number of centres: 10	Mean age (range): *32.8 (18-43** years, Mean gestational age (range): *19+6* *(8+1 - 38+6)* weeks, 1st trimester: 8 (13%).				Ravgen Inc. (USA)				
Ehrich 2011[50] USA Study start date: May 2009	Prospective case-control (T21 matched 1:11 with euploid samples) Number of centres: NR	N=480 requested from independent 3rd-party database. Pregnancies at increased risk for foetal aneuploidies with scheduled invasive diagnostic procedure (unclear if singleton or also multiple pregnancies). Median age (range): 37 (18 -47) years.	N=13 (2.7%): 9 sample volume <3.5 ml, 1 dropped, 2 mixed together, 1 tube broke during centrifugation.	T21	High risk: Positive serum screening 30.2%, AMA ≥ 35 years 68.3%, Ultrasound abnormality 12.9%, Positive family history 5.2%, Not specified 10.2%.	MPS (whole genome) Sequenom (USA)	Amniocentesis (81%) or CVS (19%) and karyotype (60%), FISH (3%), both (36%) or QF-PCR (1.6%)	None	Test performance for T21	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Median gestational age (range): 16 (8-36) weeks.								
Hall 2014[51] USA Study start date: March 2012	Nested case-control (selected from a cohort of >1000 women, all T13 cases matched 1:3 on gestational age) Number of centres: NR	N=68 (17 T13, 51 euploid) High-risk pregnancy couples, women ≥ 18 years, singleton pregnancy. Median gestational age (range): 16.0 (12.1-22.7) weeks, 1st trimester: 23 (35.9%).	N=1/>1,000 (<0.1%) from cohort: 1 known foetal mosaicism.	T13	High-risk for foetal aneuploidy (positive serum screen, ultrasound abnormality or maternal age of greater than 35 years)	SNP- and NATUS Natera Inc. (USA)	CVS, amniocentesis or genetic testing of cord blood, buccal, saliva, or products of conception	None	1) Test performance for T13 detection. 2) Specificity of T18, T21 and Monosomy X detection.	Accuracy of NIPT
Huang 2014[22] China (Denmark,	Prospective, multicentre cohort	N=189 Twin pregnancies requiring invasive procedure (CVS/	N=NR Intrauterine death, without	T21, T18	All high risk Threshold and	MPS (whole genome)	Full karyotyping from CVS	None	Test performance for T18 and T21 detection in twin	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Hong Kong) Study start date: NR	Number of centres: 7	amniocentesis) Median age (range): 31 (22-44) years. Median gestational age (range): 19 (11-36) weeks. 1st trimester: ≥ 2.1%, 2nd trimester: ≥ 74%	foetal karyotype		risk establishment NR	NIFTY test BGI-Shenzen (China)	(2.1%), amniocentesis (94.2%), or cordocentesis (3.7%)		pregnancies	
Jeon 2014[39] South Korea, China Study start date: March 2012	Prospective cohort Number of centres: 1	N=155 High-risk women scheduled for amniocentesis, ≥ 19 years old, singleton pregnancy with a gestational age of ≥ 12 weeks. Mean age (SD),	NR	T21, T18	High risk of foetal defects by standard aneuploidy screening with individual risk scores and interpretations produced by accredited clinical laboratories.	MPS (whole genome) Semiconductor sequencing	Amniocentesis and foetal karyotyping	None	T18 and T21 detection by semiconductor sequencer Ion Proton (PPV, NPV).	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		range: 30.73 (4.99), 19-43 years. Trimester: 1st: <18.1%, 2nd: >55.5%.								
Jiang 2012[23] China Study start date: June 2009	Prospective cohort Number of centres: 3	N=903 Inclusion criteria NR Age range: 20-45 years. Gestational age: 10-34 weeks (all trimesters).	Criteria NR No exclusions recorded	T21, T18 T13	Prevalence of aneuploidy suggests a general obstetric population but all women had invasive testing.	MPS (whole genome) BGI-Shenzhen (China)	Full karyotyping from amniocentesis	None	1) Aneuploidy detection. 2) GC content and sequencing bias. Relation between foetal fraction and gestational age.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Korostolev 2014[40] Russia Study start date: 2012	Prospective cohort Number of centres: NR (Moscow private clinics)	N=1,968 included, N=1,728 for NIPT. Women with singleton pregnancies, high risk for aneuploidies, >9 weeks' gestation. Mean age (range): 34.4 (26-45) years. Mean gestational age (range): 14 (9-33) weeks. 1st trimester: "about 50%".	N=240 (12.2%): Ultrasound abnormality (increased NT, heart defects, malformations, foetal growth retardation) or presence of balanced chromosomal rearrangements in the parents.	T21, T18, T13	Mixed risk: High risk result of combined FTS 87%, AMA ≥ 35 years only or women's will without any risk of chromosomal pathology 13%.	SNP and NATUS Panorama Natera Inc. (USA)	Invasive prenatal diagnosis with karyotyping or CMA (n=57), phenotypic newborn assessment (n=624), TOP and molecular study (n=1).	None	NIPT and/or invasive test based on CMA for chromosomal abnormalities diagnostics	Accuracy of NIPT
Lau 2012[24] Hong Kong, China, Japan	Prospective cohort	N=108 Pregnant women undergoing CVS or amniocentesis	NR	T21, T18, T13	Mostly high risk: Positive 1st trimester screening 47.2%,	MPS (whole genome)	Conventional karyotyping from	None	Diagnostic accuracy of novel z-score method with internal	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Study start date: NR	Number of centres: 1	(possibly singleton pregnancies but NR). Mean age (SD): 37 (4.3) years, Median gestational age (range): 12+5 (11+4 – 28+0) weeks. 1st trimester: 97 (89.8%)			positive 1st trimester sonographic markers 22.2%, other structural anomalies 1.5%, previous T21 0.9%, maternal anxiety 11.1%.	BGI-Shenzhen (China)	CVS (94.4%) or amniocentesis (5.6%)		reference chromosome.	
Lau 2014[25] Hong Kong, USA, China Study start date: August 2011	Prospective cohort Number of centres: 1	N=1,982 (1,929 singleton, 30 twin pregnancies, 23 internal control samples) Any pregnant women ≥12 weeks of gestation accepted for NIPT, regardless of whether they had undergone any	NR	T21, T18 T13	Prenatal diagnosis centre accepted referral of any pregnant woman for NIPT: Previous trisomy / Family history 53 (2.7%).	MPS (whole genome) NIFTY test BGI-Health (China)	Conventional karyotyping from CVS or amniocentesis, postnatal karyotyping or birth phenotype	None	Test accuracy for common autosomal trisomies, sex chromosomal abnormalities and other chromosome abnormalities.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		previous T21 screening. Mean age (SD), range: 36 (4.35), 20-46 years. Median gestational age: 14.5 weeks. 1st trimester: 56.25%. Ethnicity: Chinese 90.91%, Caucasian 5.21%, Other 3.88%.			No prior screening test: 669 (34.2%). Prior screening test 1,290 (65.8%): High risk 593/1,290 (46.0%), Low risk 368/1,290 (28.5%), Result not available yet 329/1,290 (25.5%).					

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Liang 2013[26] China Study start date: March 2009	Prospective cohort Number of centres: 3	N=435 High-risk pregnant women scheduled for invasive prenatal diagnostics. Mean age (SD): 31 (5.9) years. Median gestational age (range): 21+3 (11+3 – 39+3) weeks. 1st trimester: 1 (0.23%).	NR	T21, T18 T13	All high risk: AMA (≥35 years) 84 (19.3%), Positive serum screening 217 (49.9%), Ultrasound abnormality 67 (15.4%), Prior aneuploidy pregnancy 4 (0.9%), Multiple indications 63 (14.5%).	MPS (whole genome) Berry Genomics (China)	CVS (0.92%), cordocentesis (22.30%) or amniocentesis (76.78%) and full foetal karyotyping	None	Test accuracy for detection of foetal aneuploidies for all 24 chromosomes in one single sequencing event	Accuracy of NIPT
Nicolaides 2012[27] UK	Retrospective cohort of stored samples	N=2,230 original cohort, N=2,049 eligible	N=181 (8.1%): 74 no foetal karyotype,	T21, T18	General obstetric population undergoing first-trimester screening for	DANSR FORTE	86 (4.2%) CVS or amniocentesis and foetal	First-trimester CT (free β-hCG, PAPP-A, NT) with or without additional	1) Performance of screening by NIPT for	Comparison of NIPT with CT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Study start date: October 2010	Number of centres: 1	cases. Women with singleton pregnancies attending for first-trimester combined screening for aneuploidies and ultrasound (general obstetric population). Median age (IQR): 31.8 (27.7 – 35.4) years, Gestational age, range: 11+0 – 13+6 weeks, 1st trimester: 100%. Ethnicity: Caucasian 69.8%, African 20.6%,	7 abnormal karyotype other than T21 or T18, 29 inadequate sample volume, 1 wrongly labelled 70 lab mixed samples together.		aneuploidies as part of their routine antenatal care. All had 1st-trimester combined test: Median estimated T21 risk (range) 1:8,469 (1:2–1:23,527), Median estimated T18 risk (range) 1:14,894 (1:2-1:47,472).	Harmony Prenatal Test Ariosa Diagnostics (USA)	karyotyping. 1963 (95.8%) phenotypic newborn examination.	ultrasound markers (nasal bone, tricuspid regurgitation, reversed a-wave in ductus venosus). Risk threshold ≥1:150 (0.67%) for T21 and T18.	trisomies 21 and 18. 2) Comparison of NIPT with detection rate and false positive rate of 1st-trimester CT with or without additional ultrasound markers.	

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		South Asian 4.0%, East Asian 2.8%, Mixed 2.8%.								
Nicolaides 2013[28] UK Study start date: NR	Prospective cohort Number of centres: 1	N=242 Women with singleton pregnancies undergoing CVS at 11-13 weeks' gestation, ≥ 18 years, ≥ 10 weeks gestation. Mean age (range): 35.7 (18.5- 46.5) years. Median gestational age (range): 13.1 (11.3 – 13.9) weeks. 1st trimester: 100%.	NR	T21, T18, T13	High risk for aneuploidies or sickle cell disease: 1st-trimester CT >1:300 227 (93.8%), AMA 5 (2.1%), Previous aneuploidy pregnancy 6 (2.5%), Sickle cell testing 4 (1.7%). Median estimated risk for T21, T18	SNP- and NATUS Natera Inc. (USA)	CVS and karyotyping	None	Performance of NIPT to detect T21, T18, T13, SCA and triploidy.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
					or T13 by CT (range): 1:75 (1:2–1:12,433).					
Norton 2012[29] USA, Netherlands, Sweden Study start date: August 2010	Prospective, multicentre cohort study (NICE study) Number of centres: 48	N=4,002 enrolled, N=3,228 eligible: Women ≥ 18 years, gestational age ≥ 10 weeks, with singleton pregnancy, scheduled for invasive testing for any indication. Mean age (SD), range: 34.3 (6.4), 18-50 years. Mean gestational age (SD), range: 16.9 (4.1), 10-38.7 weeks.	Exclusion criteria: Multiple pregnancies, known maternal aneuploidy, active malignancy or history of metastatic cancer, already undergone CVS or amniocentesis. N=774 (19.3%): 433 samples used for assay development. 237 failed I/E	T21, T18	Undergoing invasive testing for any indication (primarily high risk women)	DANSR, FORTE Harmony Prenatal Test Ariosa Diagnostics (USA)	Karyotyping, FISH or QF-PCR from amniocentesis (74.7%) or CVS (25.3%)	None	1) Harmony Test performance for T21 and T18 at 1% risk cutoff. 2) Foetal fraction. Test performance at different risk cutoff values.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Ethnicity: Caucasian 49.6%, African American 6.4%, Asian 13.4% , Hispanic 22.7%, Other 7.9%.	criteria, 84 insufficient sample volume, 20 incorrect sample labelling.							
Norton 2015[6] USA, Sweden Study start date: March 2012	Prospective multicentre cohort (NEXT study) Number of centres: 35	N=18,955 enrolled. N=18,510 met I/E criteria. Women with singleton pregnancies, ≥ 18 years of age, presenting for aneuploidy screening at 10-14 weeks of gestation (NIPT and 1st-trimester CT). Mean age (range): 31	N=450 (2.4%): 229 did not meet inclusion criteria or met exclusion criteria, 31 had twins discovered on NT testing, 121 had unknown ovum-donor status, 64 withdrew or were withdrawn	T21, T18, T13	General obstetric population (unselected)	DANSR, FORTE Harmony Prenatal Test Ariosa Diagnostics (USA)	Invasive prenatal testing (135 CVS, 422 amniocentesis), 52 postnatal genetic testing, 16 testing on products of conception, all other examinati	First-trimester CT (cut-off ≥1:270 for T21, ≥1:150 for T18 and T13)	1) Area under ROC curve for T21 screening with NIPT versus standard screening. 2) Evaluation of NIPT and standard screening to assess the risk for T18 and T13.	Comparison of NIPT with CT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		(18-48) years. Mean gestational age (range): 12.5 (10.0-14.3) weeks. 100% 1st trimester.	by investigator.				on of the newborn.		Performance of NIPT in low-risk patients.	
Palomaki 2012[52] USA Study start date: Trial submission 6th April 2009	Nested case-control in a cohort (Part of an international clinical validation study, NCT00877292). Each pregnancy with T18 and T13 matched	N=4,664 in cohort, N=293 case-control study (62 T18, 12 T13, 219 euploid) plus 212 T21 and 1,483 matched controls reported earlier [62]. N=1,988 for NIPT. Singleton pregnancies at high risk for T21.	N=279/4,664 (6.0%) from cohort: 116 sample not adequate, 112 multiple gestation / foetal death, 51 no karyotype /outcome available. N=2,397/4,385 (54.7%):	T21, T18, T13	High risk for T21: 1st-trimester screening positive: 7.2%, 2nd-trimester screening positive: 4.4%, Integrated test positive: 10.2%, Ultrasound anomaly: 19.5%, AMA ≥ 38 years: 41.6%, 2 or more	MPS (whole genome) Sequenom Inc. (USA)	Amniocentesis (48.5%) or CVS (51.5%) and karyotyping	None	Correct identification of T21, T18 & T13	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
	with 3 controls based on the gestational age, enrolment site, race, and time in freezer (within 1 month). Number of centres: 27	Mean age (SD): 37.2 (5.0)* years. Median gestational age (range): 14.6 (9-22) weeks*. 1st trimester: 52%, 2nd trimester: 48%. Ethnicity: Caucasian 84.7%, Black 4%, Asian 5.4%, Unknown 5.4%.	Not selected for case-control study.		indications: 12.6%, Family history of aneuploidy: 3.4%, Other /unknown: 1.0%.					
Pergament 2014[30]	Prospective international multicentre cohort	N=1,064 enrolled, N=1,051 for testing (926 euploid, 67 T21,	N=13 (1.2%): 6 triploidy,	T21, T18, T13	543 (51.0%) High risk: abnormal serum screen, ultrasound	SNP- and NATUS	Amniocentesis/CVS (44.1%) and	None	Performance of single-nucleotide polymorphism	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
USA Study start date: NR	Number of centres: 36	32 T18, 14 T13, 12 Monosomy X). Singleton pregnancies of at least 7 weeks of gestation. Mean age (SD), range: 30.3 (7.4), 18-47 years. Mean gestational age (SD), range: 17.0 (4.1), 7.6-40.6 weeks.	3 foetal mosaic, 2 47,XXY, 1 47,XXX, 1 47,XYY.		abnormality, maternal age ≥ 35 years. 521 (49.0%) Low risk: maternal age < 35 years and lacking any reported high-risk indications.	Natera Inc. (USA)	karyotyping/FISH; genetic testing of cord blood, buccal sample or saliva (13.2%) or products of conception (42.8%).		-based test on both high- and low-risk pregnant women.	
Porreco 2014[31] USA Study start date:	Prospective multicentre cohort (NCT00847990) Number of	N=4,170 enrolled, N=3,430 for testing. Singleton pregnancies, high risk for foetal aneuploidy	N=740 (17.7%): 320 insufficient sample volume, 120 outside 6h lab processing window,	T21, T18, T13	High risk for foetal aneuploidy: Abnormal NT 104 (3%), Abnormal Triple/ quad screen 289	MPS (whole genome) MaterniT21 ® PLUS	Amniocentesis (75.5%) or CVS (24.5%) and karyotype	None	Clinical performance of MPS to test for T21, T18, T13, foetal sex and SCA.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
September 2009	centres: 31	undergoing invasive procedure. Mean age (SD), range: 35.1 (5.6), 18-50 years. Mean gestational age (SD), range: 16.3 (3.5), 9.0-37.0 weeks. Ethnicity: White 60.1%, Asian 18.7%, Hispanic or Latino 9.9%, Black 4.5%, Multiple 5.5%.	270 used as lab quality control set, 24 incomplete case report forms, 6 no amniocentesis / CVS.		(8.4%), Abnormal ultrasound 492 (14.3%), AMA ≥ 35 years 1,417 (41.3%), Multiple indications 929 (27.1%), Previous or family history of aneuploidies 98 (2.9%).	Sequenom, Inc. (USA)				
Quezada	Prospective	N=2,905	N=NR	T21, T18,	No prior screening, general	DANSR,	CVS or amniocent	First-trimester CT for T21	1) Numbers and	Comparison of

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
2015[41] UK Study start date: October 2012	cohort Number of centres: 1	Women with singleton pregnancies undergoing routine first-trimester screening for the major trisomies by NIPT and by the combined test. Mean age (range): 36.9 (20.4–51.9) years. Median gestational age (range): 10+4 (10+0 -11+6) weeks. 1st trimester: 100%. Ethnicity: Caucasian 2,570 (88.5%), South Asian 173		T13	obstetric population, AMA ≥ 35 years 1,958 (67.4%).	FORTE Harmony Ariosa Diagnostics (USA)	esis and foetal karyotyping, post-mortem examination and karyotyping, newborn phenotype	(PAPP-A, free β-hCG, nuchal translucency) Risk threshold ≥ 1/100 for T21.	concordance of results of NIPT and 1st-trimester combined screen. 2) Discordant results between NIPT and foetal karyotype.	NIPT with CT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		(6.0%), East Asian 96 (3.3%), Afro-Caribbean 21 (0.7%), Mixed 45 (1.5%).								
Sago 2014[42] Japan Study start date: April 2013	Prospective multicentre cohort Number of centres: 15 in April 2013, 37 by March 2014	N=7,740 Women with singleton pregnancies, 10 to 18 weeks' gestation, high-risk for aneuploidy, requesting NIPT. Mean age (range): 38.3 (21-48) years. Mean gestational age (range): 13.3 (10.0-19.9) weeks.	Multiple Pregnancy N=NR	T21, T18, T13	All high-risk: Maternal age ≥ 35 years 7387 (95.4%), Prior history 226 (2.9%), Ultrasound abnormality 108 (1.4%), Serum marker 16 (0.2%), Balanced Robertsonian translocation 3 (0.04%).	MPS (whole genome) MaterniT21 PLUS Sequenom Inc. (USA)	CVS or amniocentesis and foetal karyotyping, foetal death and karyotyping or birth phenotype	None	PPV for T21, T18 and T13.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		1st and 2nd trimester.								
Sehnert 2011[53] USA Study start date: April 2009	<u>Training set:</u> Prospective case-control (all foetuses with abnormal karyotype as well as a random selection of non-affected individuals) <u>Validation set:</u> Prospective case-control or case series Number of centres: 13	N=1,014 in cohort, 946 singleton pregnancies with foetal karyotype. Mean age (SD), range: 35.6 (5.66), 17-47 years. Mean gestational age (range): 15+4 (6+1 - 38+1) weeks. Trimester NR. Ethnicity: 62.7% Caucasian 16.5% Hispanic 6.2% Asian,	N=68/1,014 (6.7%) from cohort: Unspecified From training set N=6 (8.5%): 4 twin gestations, 1 contaminated during preparation, 1 69,XXX. From validation set N=1 (2.1%): 1 twin gestation.	T21, T18, T13	906/946 (96%) showed at least 1 clinically recognized risk factor for aneuploidy: AMA ≥35 years 52.1%, Screen positive 18.6%, Increased NT 4.5%, Other congenital abnormality 9.0%, Other maternal risk 7.4%.	MPS (whole genome) Verinata Health (USA)	CVS or amniocentesis and foetal karyotype	None	Test performance for T21, T18, T13, gender and Monosomy X classification	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		5.2% multi-ethnic Selected for training set: 71/435, Selected for validation set: 48/575.								
Shaw 2014[32] Taiwan, China Study start date: June 2012	Prospective cohort Number of centres: 11	N=201 Pregnant women > 12 weeks' gestation. <u>High risk</u> (n=100): Mean age (SD): 35.1 (3.2) years. Mean gestational age (SD) 17.3 (2.1) weeks. 98 singleton, 2 twin pregnancies.	N=1 (0.5%): 1 due to early gestational age (<12 weeks)	T21, T18, T13	Very high risk (T21 risk >1:30 or NT >3.0mm): N=100 Average screening risk: 1:22.8. Low risk (T21 risk <1:1,500): N=100 Average screening risk:	MPS (whole genome) Berry Genomics (China)	Amniocentesis and karyotyping or birth outcome	None	Test performance for detection of all foetal autosomal and sex chromosome aneuploidies	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Low risk (n=100): Mean age (SD): 34.6 (2.6) years. Mean gestational age (SD) 16.1 (3.0) weeks. 98 singleton, 2 twin pregnancies.			1:3,179.					
Song 2013[33] China Study start date: April 2011	Prospective cohort Number of centres: 2	N=1,916 Singleton pregnancies, women <35 years undergoing routine antenatal screening. Mean age (SD), range: 29.03 (2.7), 20 - 34 years.	N=NR	T21, T18 T13	General obstetric population < 35 years. High risk 275/1,741 (15.8%): Positive serum screening >1:270: 249 (14.3%),	MPS (whole genome) Berry Genomics (China)	CVS, amniocentesis or cordocentesis and karyotyping or birth phenotype	2nd trimester triple serum screening (α-fetoprotein, free β-hcg, unconjugated estriol) Cutoff ≥ 1:270 for T21 and T18.	NIPT test performance for detection of T21, T18, T13 and SCA. Comparison of NIPT and serum screening performance.	Comparison of NIPT with CT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Mean gestational age (SD), range: 16.57 (1.56), 11 - 21+6 weeks. 1st trimester: 3.4%, 2nd trimester: 96.6%. Assisted conception 14 (0.8%).			Increased NT: 10 (0.6%), Other indications 16 (0.9%). Low risk 1,466/1,741 (84.2%).					
Song 2015[45] China Study start date: May 2012	Prospective cohort Number of centres: 1	N=213 Women with singleton pregnancies, ≥ 35 years, 8+0 – 12+6 weeks' gestation, high-risk of foetal aneuploidies, presenting for NIPT. Mean age (range):	N=1 (0.5%): 1 with quality control failure (haemolysis)	T21, T18, T13	All high-risk for foetal aneuploidies due to advanced maternal age ≥ 35 years.	MPS (whole genome) Berry Genomics (China)	CVS or amniocentesis and karyotyping (n=178) or newborn phenotypic examination (n=34).	None	1) Clinical performance of NIPT in the first trimester. 2) Relationship between foetal DNA fraction and early gestational	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		37.25 (35-45) years. Mean gestational age (range): 9+6 (8+0 – 12+6) weeks. 100% 1st trimester.							age.	
Sparks 2012[54] USA Study start date: NR	Prospective case-control Number of centres: NR	Number enrolled unclear. Singleton pregnancies, women ≥ 18 years, ≥10 weeks' gestation, high risk for foetal trisomies undergoing invasive testing. Subset of N=338 (250 euploid, 72 T21, 16 T18) randomised into <u>Validation set</u>	NR	T21, T18	High risk for foetal trisomy	DANSR and z statistic or FORTE Aria Diagnostics (USA)	Invasive testing with FISH and/or karyotype analysis	None	Detecting foetal aneuploidy using DANSR and z statistic or FORTE	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		(n=167) (36 T21, 8 T18, 123 euploid): Mean age (SD), range: 33.5 (7.1), 18-51 years. Mean gestational age (SD), range: 18.6 (4.0), 11.0-36.1 weeks. <u>Training set</u> (n=171) (36 T21, 8 T18, 127 euploid): Mean age (SD), range: 34.5 (6.3), 18-44 years. Mean gestational age (SD), range: 17.6 (4.4), 10.3-33.0								

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		weeks.								
Stumm 2014[34] Germany , Switzerland Study start date: NR	Prospective cohort Number of centres: 5	N=522 recruited, N=504 for testing. Women with singleton pregnancy, ≥18 years, high risk for aneuploidies, with foetal karyotype. Mean age (range): 36.0 (19-47) years. Mean gestational age (range): 15.6 (11+0 – 32+1) weeks.	N=18 (3.4%): 9 no consent, 8 no karyotype, 1 sample previously tested.	T21, T18 T13	All high risk for chromosomal aberrations: AMA >35 years 69.5%, Positive serum markers 11.1%, Ultrasound abnormality 39.3%, Family history 2.1%, Parental chromosome abnormality 0.4%, Other 14.9% (more than 1 risk factor in 179/522)	MPS (whole genome) LifeCodexx (Germany)	Amniocentesis, CVS, cordocentesis and foetal karyotyping	None	1) Diagnostic accuracy for foetal T21 detection (using DAP.21). 2) Diagnostic accuracy for foetal T13 and T18 detection (using DAP.plus) and comparison of algorithms for T21.	Accuracy of NIPT
Verweij	Multicentre	N=595 enrolled,	N=75 (12.6%):	T21	91.2% increased risk for T21 based	DANSR	CVS (54%) or	None	Test performance	Accuracy

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
2013[35] Netherlands, Norway, Sweden, USA Study start date: May 2011	international prospective cohort (EU-NITE study) Number of centres: 6 (4 Dutch, 2 Swedish)	N=520 eligible. Women undergoing invasive testing, singleton pregnancy, ≥10 weeks' gestation. Mean age (SD), range: 36.4 (4.6), 20-47 years. Mean gestational age (SD), range: 14.0 (2.1), 10-28 weeks. Ethnicity: Caucasian 84.8%, Mediterranean 6.0%, Asian 3.3%,	21 failed I/E criteria (non-invasive procedure performed, twin pregnancy, no blood sample); 19 insufficient plasma volume; 11 logistical problems - shipping difficulties; 24 chromosome abnormalities other than T21.		on 1st trimester screening (serum screening, NT and/or maternal age), detection of foetal anomalies on ultrasound, previous affected pregnancy or family history. 8.8% other indications (psychosocial or anxiety reasons).	FORTE Harmony Ariosa Diagnostics (USA)	amniocentesis (46%) and karyotyping or quantitative fluorescent PCR		for T21 detection by shipping whole blood samples from Europe to a laboratory in the USA.	of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		Black 1.3%, Other 4.6%.								
Wax 2015[43] USA Study start date: June 2012	Retrospective review of prospective cohort Number of centres: 1	N=1,046 eligible for NIPT, N=166 high-risk pregnant women with singleton pregnancies opted for NIPT. Mean age (SD): 34.6 (5.5) years. Gestational age: range 10+0 – 21+6 weeks. 1st and 2nd trimester.	Multiple pregnancy N=NR; N=880 (84.1%) chose not to have NIPT.	T21, T18, T13	All high-risk: AMA ≥ 35 years 742 (70.9%), Ultrasound abnormality 280 (26.8%), Positive screen 115 (11.0%), Prior trisomy 15 (1.4%), Parental translocation 1 (0.1%).	MPS (whole genome) Manufacturer: NR	Amniocentesis (n=56) or CVS (n=50) and karyotyping, postnatal karyotyping of neonatal blood, birth phenotype from records	None	Difference in genetic counselling utilisation, invasive procedures and T21 detection before and after NIPT implementation.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
Zhang 2015[5] China, Hong Kong (Denmark) Study start date: January 2012	Prospective multicentre cohort Number of centres: 508	N=147,314 samples received for NIPT. N=147,103 appropriate samples. Women with singleton or twin pregnancy, ≥ 9 weeks of gestation, ≥ 18 years old. Mean age (range): 30.9 (18-56) years. Mean gestational age (range): 18.7 (9-37) weeks. Trimester: 1st (9-13 wks): 4.21%, 2nd (14-27 wks): 94.13%,	N=211 (0.14%): 211 samples rejected due to inadequate volume, contamination, <9 gestational weeks, or improper labelling.	T21, T18, T13	Mixed (high-risk, low-risk or no prior screening): Positive T21 screening 37.83%, Negative T21 screening 21.43%, No prior screening 40.73%. AMA 23.04%, Family history of aneuploidies 0.01%, Sonographic markers of chromosomal abnormality 1.61%.	MPS (whole genome) NIFTY test BGI-Health (China)	Karyotyping or clinical follow-up results.	None	1) Clinical performance of NIPT in detecting T21, T18, and T13. 2) NIPT performance in twin pregnancies. NIPT performance for T21 detection in high-risk and low-risk subjects. Factors contributing to NIPT false-positive and false-negative results.	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
		3rd (≥ 28 wks): 1.47%, Unknown: 0.18%. 99.45% singletons, 0.55% twins.								
Zhou 2014[44] China Study start date: November 2011	Prospective cohort Number of centres: 1	N=7,705 Women with singleton pregnancies, 12-24 weeks' gestation, high-risk or no prior T21 screening. Gestational age: 12-24 weeks. 1st and 2nd trimester.	Multiple pregnancy N=NR	T21, T18, T13	Mixed risk: AMA ≥ 35 years: 40.4%, High risk T21 screening: 32.1%, Low risk T21 screening: 11.3%, No prior T21 screening: 56.6%.	MPS (whole genome) NIFTY test BGI-Shenzen, China	Amniocentesis and karyotyping (n=54), postnatal karyotype (n=2) or birth outcome (n=3,894).	None	1) NIPT performance for detection of trisomies 13, 18, and 21. 2) Confirming care flow path	Accuracy of NIPT
Zimmermann 2012[55]	Prospective case-control	N=166 (11 T21, 3 T18, 2	NR	T21, T18 T13	Mixed: Aneuploidy	SNP-based, Parental Support (PS)	Invasive testing and FISH	None	Detection of foetal aneuploidies	Accuracy of NIPT

Reference	Study design	Participants	Exclusions from study	Trisomies investigated (T21, T18, T13)	Prior risk	Type of test	Reference method	Comparator if applicable	Primary / secondary outcome	Accuracy of NIPT or comparison of NIPT with CT
USA Study start date: NR	Unblinded proof-of-principle study Number of centres: NR	T13, 2 45X, 2 47XXY, 146 putatively euploid) Singleton pregnancies, women ≥ 18 years, ≥ 9 weeks' gestation. Median gestational age: 17.0 and 17.5 weeks for euploid and aneuploid samples, respectively.			samples from pregnant women with invasive prenatal testing. Putative euploid samples from average-risk women without known risk indicators.	algorithm Natera Inc. (USA)	and/or karyotype in aneuploid samples, 62/146 putative euploid samples comfirmed by karyotyping of post-birth child tissue.		at chromosomes 13, 18, 21, X, and Y.	

AMA, advanced maternal age; β-hCG, β-fragment of human chorionic gonadotropin; CMA, chromosomal microarray; CT, first-trimester combined test; CVS, chorionic villus sampling; DANSR, digital analysis of selected regions; DNA, deoxyribonucleic acid; FISH, fluorescence in situ hybridisation; FORTE, Foetal fraction Optimized Risk of Trisomy Evaluation; FTS, first-trimester combined test; ICD, international classification of diseases; I/E criteria, inclusion or exclusion criteria; IQR, interquartile range; IVF, in vitro fertilisation; MPS, massively parallel sequencing; MS-AFP, maternal serum alpha-fetoprotein; NATUS, Next Generation Aneuploidy Test Using SNPs; NIFTY, Non-invasive Fetal Trisomy Test; NIPT, non-invasive prenatal testing; NPV, negative predictive value; NR, not reported; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein; PCR, polymerase chain reaction; PPV, positive predictive value; QF-PCR, quantitative fluorescent polymerase chain reaction; ROC, receiver-operating-characteristic curve; SCA, sex chromosome anomalies; SD, standard deviation; SNP, single-nucleotide polymorphism; TOP, termination of pregnancy. * Reviewer calculation from published data.