

Supplement 4 Table of excluded studies with reason

Reference	Reason for exclusion
1. Anonymous. Cell-free fetal DNA tests for trisomy show promise in women at lower risk of affected pregnancies: lower rates of false-positive returns, higher positive predictive value are associated with cfDNA tests versus standard screening panels, say experts. <i>Am J Med Genet A</i> 2014;164A(6):viii-ix.	Commentary
2. Anonymous. Trisomy 21 DNA test (MaterniT21) for detecting Down syndrome in the first trimester. <i>Manag Care</i> 2012;21(4):19-20.	Commentary
3. Ashoor G, Syngelaki A, Wang E, Struble C, Oliphant A, Song K, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. <i>Ultrasound Obstet Gynecol</i> 2013;41(1):21-5. 269	Case control studies: <15 cases
4. Bianchi DW, Lamar Parker R, Wentworth J, Madankumar R, Saffer C, Das AF, et al. DNA sequencing versus standard prenatal aneuploidy screening. <i>Obstetrical and Gynecological Survey</i> . 2014;69(6):319-21.	Editorial
5. Canick, J.A., et al., DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. <i>Prenatal Diagnosis</i> , 2012. 32(8): p. 730-4.	Nested case-control study: < 15 cases
6. Chiu, R.W., et al., Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. <i>Proceedings of the National Academy of Sciences of the United States of America</i> , 2008. 105(51): p. 20458-63.	Case-control study: < 15 cases
7. Dar P, Curnow KJ, Gross SJ, Hall MP, Stosic M, Demko Z, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. <i>Am J Obstet Gynecol</i> . 2014;211(5):527.e1-e17.	Incomplete 2x2 table; index test results used as inclusion criteria so incomplete 2x2 table
8. Deng, Y.H., et al., Non-invasive prenatal diagnosis of trisomy 21 by reverse transcriptase multiplex ligation-dependent probe amplification. <i>Clinical Chemistry & Laboratory Medicine</i> , 2011. 49(4): p. 641-6.	Not cff DNA (cell-free fetal RNA)
9. Dugo N, Padula F, Mobili L, Brizzi C, D'Emidio L, Cignini P, et al. Six consecutive false positive cases from cell-free fetal DNA testing in a single	Case series: < 15 cases

referring centre. Journal of Prenatal Medicine. 2014;8(1-2):31-5.	
10. Faas, B.H., et al., Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. Expert Opinion on Biological Therapy, 2012. 12 Suppl 1: p. S19-26.	Case series: < 40 women
11. Fairbrother, G., et al., Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. Prenatal Diagnosis, 2013. 33(6): p. 580-583.	No reference standard results
12. Fang Y, Wang G, Wang C, Suo F, Gu M, Xia Y. The Diagnosis Pattern of Mid-Trimester Fetal Chromosomal Aneuploidy in Xuzhou and the Clinical Applications. Cell biochemistry and biophysics. 2015.	Incomplete 2x2 table; unclear reporting, incomplete follow up of cffDNA testing negative cases
13. Feenstra, H., et al., Complexity of noninvasive prenatal screening and diagnostic testing for an unbalanced translocation involving chromosomes 5 and 18. Prenatal Diagnosis, 2014. 34: p. 195-198.	Case report
14. Futch T, Spinoza J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. Prenat Diagn. 2013;33(6):569-74.	Incomplete 2x2 table; index test results used as inclusion criteria so incomplete 2x2 table
15. Ghanta, S., et al., Non-invasive prenatal detection of trisomy 21 using tandem single nucleotide polymorphisms. PLoS ONE [Electronic Resource], 2010. 5(10): p. e13184.	Case-control study: < 15 cases
16. Gil, M.M., et al., Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. Ultrasound in Obstetrics & Gynecology, 2013. 42(1): p. 34-40.	Cohort study: < 50 women with index and reference test result
17. Grati, F.R., et al., Fetoplacental mosaicism: potential implications for false-positive and false-negative noninvasive prenatal screening results. Genetics in Medicine, 2014. 16(8): p. 620-4.	Not cff DNA (cytogenetic material from CVS/Amnio)
18. Gromminger, S., et al., Fetal aneuploidy detection by cell-free DNA sequencing for multiple pregnancies and quality issues with vanishing twins. Journal of Clinical Medicine, 2014. 3(3): p. 679-692.	Cohort study: < 50 women
19. Guex, N., et al., A robust second-generation	Letter

	genome-wide test for fetal aneuploidy based on shotgun sequencing cell-free DNA in maternal blood. <i>Prenatal Diagnosis</i> , 2013. 33: p. 707-710.	
20.	Guo, Q., et al., Simultaneous detection of trisomies 13, 18, and 21 with multiplex ligation-dependent probe amplification-based real-time PCR. <i>Clinical Chemistry</i> , 2010. 56(9): p. 1451-9.	Participants not pregnant women
21.	Hayes Inc., Harmony Prenatal Test (Structured abstract). <i>Health Technology Assessment Database</i> , 2012.	Abstract of review
22.	Hayes Inc., Noninvasive Prenatal Testing (NIPT) for fetal aneuploidy (Structured abstract). <i>Health Technology Assessment Database</i> , 2013.	Abstract of review
23.	Hill, M., et al., Evaluation of non-invasive prenatal testing (NIPT) for aneuploidy in an NHS setting: a reliable accurate prenatal non-invasive diagnosis (RAPID) protocol. <i>BMC Pregnancy & Childbirth</i> , 2014. 14: p. 229.	Protocol, no data presented
24.	Hyett J. Non-invasive prenatal testing for down syndrome. <i>Australian Prescriber</i> . 2014;37(2):51-5.	Review
25.	Jensen TJ, Zwiefelhofer T, Tim RC, Dzakula Z, Kim SK, Mazloom AR, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. <i>PLoS ONE</i> . 2013;8(3):e57381.	Re-uses some of the same samples as Palomaki et al. (2012); excluded to prevent double counting
26.	Jorgez, C.J., et al., Elevated levels of total (maternal and fetal) beta-globin DNA in maternal blood from first trimester pregnancies with trisomy 21. <i>Human Reproduction</i> , 2007. 22(8): p. 2267-72.	Measurement of total blood DNA levels
27.	Juneau K, Bogard PE, Huang S, Mohseni M, Wang ET, Ryvkin P, et al. Microarray-based cell-free DNA analysis improves noninvasive prenatal testing. <i>Fetal Diagn Ther</i> . 2014;36(4):282-6.	Reference standard not fetal karyotyping or postnatal phenotype
28.	Kagan KO, Wright D, Nicolaides KH. First-trimester contingent screening for trisomies 21, 18 and 13 by fetal nuchal translucency and ductus venosus flow and maternal blood cell-free DNA testing. <i>Ultrasound in obstetrics & gynecology: the official journal of the International Society of Ultrasound in Obstetrics and Gynecology</i> . 2014.	Modelled data
29.	Lambert-Messerlian G, Kloza EM, Williams IJ, Loucky J, O'Brien B, Wilkins-Haug L, et al. Maternal plasma DNA testing for aneuploidy in pregnancies achieved by assisted reproductive technologies. <i>Genetics in Medicine</i> .	No additional diagnostic accuracy data to Palomaki 2011[62]

2014;16(5):419-22.	
30. Larion S, Warsof SL, Romary L, Mlynarczyk M, Peleg D, Abuhamad AZ. Uptake of noninvasive prenatal testing at a large academic referral center. <i>American Journal of Obstetrics & Gynecology</i> . 2014;211(6):651.e1-7.	No diagnostic accuracy data
31. Lee da, E., et al., Non-invasive prenatal testing of trisomy 18 by an epigenetic marker in first trimester maternal plasma. <i>PLoS ONE [Electronic Resource]</i> , 2013. 8(11): p. e78136.	Nested case-control study: < 15 cases
32. Levy B, Norwitz E. Non-invasive prenatal aneuploidy testing: technologies and clinical implication. <i>MLO Med Lab Obs</i> 2013;45(6):8, 10, 12 passim; quiz 16.	Review
33. Liao C, Yin AH, Peng CF, Fu F, Yang JX, Li R, et al. Noninvasive prenatal diagnosis of common aneuploidies by semiconductor sequencing. <i>Proc Natl Acad Sci U S A</i> . 2014;111(20):7415-20.	Incomplete 2x2 table; used cross-validation method to evaluate sensitivity and specificity so no 2 x 2 table
34. Lim, J.H., et al., Disease specific characteristics of fetal epigenetic markers for non-invasive prenatal testing of trisomy 21. <i>BMC Medical Genomics [Electronic Resource]</i> , 2014. 7: p. 1.	Method development study
35. Lim, J.H., et al., Non-invasive detection of fetal trisomy 21 using fetal epigenetic biomarkers with a high CpG density. <i>Clinical Chemistry & Laboratory Medicine</i> , 2014. 52(5): p. 641-7.	Nested case-control study: < 15 cases
36. Lim, J.H., et al., Non-invasive epigenetic detection of fetal trisomy 21 in first trimester maternal plasma. <i>PLoS ONE [Electronic Resource]</i> , 2011. 6(11): p. e27709.	Epigenetic approach
37. Lo KK, Bousted C, Chitty LS, Plagnol V. RAPIDR: an analysis package for non-invasive prenatal testing of aneuploidy. <i>Bioinformatics</i> . 2014;30(20):2965-7.	No information on population and reference standard
38. Louis-Jacques, A., et al., Effect of commercial cell-free fetal DNA tests for aneuploidy screening on rates of invasive testing. <i>Obstetrics & Gynecology</i> , 2014. 123 Suppl 1: p. 67S.	Abstract
39. Louis-Jacques, A., et al., Use of commercial tests for aneuploidy screening using cell-free fetal DNA in clinical practice. <i>Obstetrics & Gynecology</i> , 2014. 123 Suppl 1: p. 154S.	Conference abstract
40. Manegold-Brauer, G., et al., A new era in prenatal care: non-invasive prenatal testing in Switzerland. <i>Swiss Medical Weekly</i> , 2014. 144: p. w13915.	Cohort study: < 50 women

41. McCullough RM, Almasri EA, Guan X, Geis JA, Hicks SC, Mazloom AR, et al. Non-invasive prenatal chromosomal aneuploidy testing--clinical experience: 100,000 clinical samples. <i>PLoS ONE</i> . 2014;9(10):e109173.	Incomplete 2x2 table; no reasonable estimate for FN or FP in 2x2 table. Reliant on clinicians reporting results back to the company on an ad-hoc basis
42. Nicolaides, K.H., et al., First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. <i>Fetal Diagnosis & Therapy</i> , 2014. 35(3): p. 185-92.	No diagnostic accuracy data
43. Nicolaides, K.H., et al., Prenatal detection of fetal triploidy from cell-free DNA testing in maternal blood. <i>Fetal Diagnosis & Therapy</i> , 2014. 35(3): p. 212-7.	NIPT for triploidy
44. Norton ME, Jelliffe-Pawlowski LL, Currier RJ. Chromosome abnormalities detected by current prenatal screening and noninvasive prenatal testing. <i>Obstetrics & Gynecology</i> . 2014;124(5):979-86.	No diagnostic accuracy data
45. O'Brien BM, Kloza EM, Halliday JV, Lambert-Messerlian GM, Palomaki GE. Maternal plasma DNA testing: experience of women counseled at a prenatal diagnosis center. <i>Genetic Testing & Molecular Biomarkers</i> . 2014;18(10):665-9.	No diagnostic accuracy data
46. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrlich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. <i>Genet Med</i> . 2011;13(11):913-20.	Uses the same samples as Palomaki et al. (2012); excluded to prevent double counting
47. Papageorgiou, E.A., et al., Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. <i>Nature Medicine</i> , 2011. 17(4): p. 510-3.	Case-control study: < 15 cases
48. Pettit KE, Hull AD, Korty L, Jones MC, Pretorius DH. The utilization of circulating cell-free fetal DNA testing and decrease in invasive diagnostic procedures: an institutional experience. <i>Journal of Perinatology</i> . 2014;34(10):750-3.	No diagnostic accuracy data
49. Platt LD, Janicki MB, Prosen T, Goldberg JD, Adashek J, Figueiroa R, et al. Impact of noninvasive prenatal testing in regionally dispersed medical centers in the United States. <i>American Journal of Obstetrics & Gynecology</i> . 2014;211(4):368.e1-7.	No diagnostic accuracy data
50. Rabinowitz, M., et al., Noninvasive aneuploidy detection by multiplexed amplification and sequencing of polymorphic Loci. <i>Obstetrics & Gynecology</i> , 2014. 123 Suppl 1: p. 167S.	Conference abstract

51. Shaw, S.W., C.P. Chen, and P.J. Cheng, From Down syndrome screening to noninvasive prenatal testing: 20 years' experience in Taiwan. Taiwanese Journal of Obstetrics & Gynecology, 2013. 52(4): p. 470-4.	Review
52. Shea JL, Diamandis EP, Hoffman B, Lo YM, Canick J, van den Boom D. A new era in prenatal diagnosis: the use of cell-free fetal DNA in maternal circulation for detection of chromosomal aneuploidies. <i>Clin Chem</i> 2013;59(8):1151-9.	Interview
53. Shi X, Zhang Z, Cram DS, Liu C. Feasibility of noninvasive prenatal testing for common fetal aneuploidies in an early gestational window. <i>Clinica Chimica Acta</i> . 2015;439:24-8.	Cohort study: < 50 women with index and reference test result
54. Skinner, J., et al., Analysis of fetal DNA in the maternal venous blood for abnormalities of chromosomes 13, 16, 18 and 21 in first-trimester spontaneous miscarriage. <i>Journal of Obstetrics & Gynaecology</i> , 2003. 23(3): p. 228-32.	Maternal plasma samples after first trimester spontaneous miscarriage vs. genetic analysis of evacuated products of the uterus
55. Sparks AB, Wang ET, Struble CA, Barrett W, Stokowski R, McBride C, et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. <i>Prenat Diagn</i> . 2012;32(1):3-9.	Incomplete 2x2 table; no reference standard for cffDNA testing negative cases
56. Struble CA, Syngelaki A, Oliphant A, Song K, Nicolaides KH. Fetal fraction estimate in twin pregnancies using directed cell-free DNA analysis. <i>Fetal Diagnosis & Therapy</i> . 2014;35(3):199-203.	No diagnostic accuracy data
57. Stumm, M., et al., Noninvasive prenatal detection of chromosomal aneuploidies using different next generation sequencing strategies and algorithms. <i>Prenatal Diagnosis</i> , 2012. 32(6): p. 569-77.	Method development
58. Tong, Y.K., et al., Noninvasive prenatal detection of fetal trisomy 18 by epigenetic allelic ratio analysis in maternal plasma: Theoretical and empirical considerations. <i>Clinical Chemistry</i> , 2006. 52(12): p. 2194-202.	Case series: < 50 women
59. Tong, Y.K., et al., Noninvasive prenatal detection of trisomy 21 by an epigenetic-genetic chromosome-dosage approach. <i>Clinical Chemistry</i> , 2010. 56(1): p. 90-8.	Case-control study: < 15 cases
60. Tsaliki, E., et al., MeDIP real-time qPCR of maternal peripheral blood reliably identifies trisomy 21. <i>Prenatal Diagnosis</i> , 2012. 32(10): p. 996-1001.	Epigenetic approach
61. van den Oever, J.M., et al., Single molecule	Case control: < 15 cases

sequencing of free DNA from maternal plasma for noninvasive trisomy 21 detection. Clinical Chemistry, 2012. 58(4): p. 699-706.	
62. van den Oever, J.M., et al., Successful noninvasive trisomy 18 detection using single molecule sequencing. Clinical Chemistry, 2013. 59(4): p. 705-9.	Case control: < 15 cases
63. Wang JC, Sahoo T, Schonberg S, Kopita KA, Ross L, Patek K, et al. Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases. Genet Med. 2014.	Incomplete 2x2 table; index test results used as inclusion criteria so incomplete 2x2 table
64. Willems PJ, Dierickx H, Vandenakker E, Bekedam D, Segers N, Deboulle K, et al. The first 3,000 Non-Invasive Prenatal Tests (NIPT) with the Harmony test in Belgium and the Netherlands. Facts views vis. 2014;6(1):7-12.	Incomplete 2x2 table; incomplete follow up of cffDNA testing negative cases
65. Wu, D., et al., Prenatal diagnosis of Down syndrome using cell-free fetal DNA in amniotic fluid by quantitative fluorescent polymerase chain reaction. Chinese Medical Journal, 2014. 127(10): p. 1897-901.	Not cff DNA (amniotic fluid)
66. Yu SC, Chan KC, Zheng YW, Jiang P, Liao GJ, Sun H, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. Proc Natl Acad Sci U S A. 2014;111(23):8583-8.	Re-uses the same samples as Chen et al. (2011) and Chiu et al. (2011); excluded to prevent double counting
67. Zhang, M., et al., Non-invasive prenatal diagnosis of trisomy 21 by dosage ratio of fetal chromosome-specific epigenetic markers in maternal plasma. Journal of Huazhong University of Science and Technology. Medical Sciences, 2011. 31(5): p. 687-92.	Epigenetic approach