International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 01 Issue 04 June 2021

Page No : 37-42

DOI: https://doi.org/10.47191/ijmscrs/v1-i4-01

Potential Use of Cell Free Fetal DNA at 13 Short Tandem Repeats Loci for Noninvasive Prenatal Paternity Test

Citra Manela[1], Rika Susanti[2], Taufik Hidayat[3]

[1,2,3]Forensic Department, Faculty of Medicine, Andalas University, Padang 25163, Indonesia

ABSTRACT

Background: Prenatal paternity test is mostly performed by using Amniocentesis or Chorionic Villus Sampling (CVS) methods. However, these methods require invasive procedures, which are potentially harmful for both the mother and the fetus. Currently, the invention of of Cell-Free Fetal DNA (cffDNA) has offered the opportunity of performing prenatal paternity test non-invasively.

Materials and Methods: This study is a cross sectional descriptive study to detect cell free fetal DNA at 13 STR loci and at amelogenin gene to evaluate fetus gender, which will be compared to the baby gender afterbirth. Healthy third semester pregnant women were included as participants. Inform consent for both the mother and the biological father has been provided.

Result: Four participants has been evaluated. In this study, in all participants, we found the presence of cffDNA in almost all of the STR loci. Some loci cannot be detected due to the small amount of cffDNA in the loci. All fetus genders detected by cffDNA in the amelogenin gene macthed the gender of the four babies afterbirth.

Conclusion: The use of Cell-Free Fetal DNA (cffDNA) is a potential non-invasive methods in prenatal paternity test. Additionally, the ability of the method to evaluate fetus gender has been suggested.

ARTICLE DETAILS

Published On:
02 June 2021

Available on:
https://ijmscr.org

KEYWORDS: prenatal paternity tests, noninvasive, cell free fetal DNA

INTRODUCTION

During pregnancy, the fetal and maternal circulation is separated by the placental membrane. The placental membrane forms an impenetrable barrier between the mother's and child's blood. However, various evidences have pointed to the incompleteness of these barriers to cellular exchange.[1] Several hypotheses regarding the origin of fetal DNA in the maternal circulation are fetal hemapoetic cells, placental trophoblast and transferred directly from the maternal. One possible explanation is that fetal DNA comes from fetal cells that were damaged when trying to enter the maternal circulation. Another hypothesis states that a high enough fetal DNA concentration is thought to originate from apoptotic placental trophoblasts, so that the concentration of fetal DNA increases with the development of the placenta during pregnancy.[2]

Fetal free DNA is the nucleus of DNA. The presence of core DNA in fetal free indicates the content of DNA satellites in which there is a short tandem repeat (STR). STR contained in fetal free DNA is a fusion of DNA / STR derived from father and mother according to Mendel's law. Micro-satellite repeat often referred to as STR is a DNA region with 3-7 bp repeat units. So in other words, STR is tandem repeat with a very short repeat motive. At each STR locus, each individual has a DNA fragment, each of which comes from its parents. The variation in the sequence of bases in the DNA within each human cell is called polymorphism. Polymorphism is a term used to indicate the existence of a different form of the same basic structure. A new locus is said to be a polymorphism if the most frequent allele is found to be less than 99% of the frequency at the locus concerned, whereas according to Hardy Weinberg's law at least 2% of the population must be heterozygous at that locus. The

nature of this polymorphism is thought to be due to unequal exchanges in the processes of mitosis and meiosis, so that it shows the variation of each individual which can provide an advantage because it distinguishes one person from another. This is used in forensic medicine as the basis for identification through polymorphic DNA analysis. The The Federal Bureau of Investigation (FBI) has its own DNA examination standards, where the National Police also uses the same standards as the FBI, as a DNA-based identification technique. This FBI standard is known as CODIS or Combined DNA Index System, using the STR (Short Tandem Repeat) method. The FBI released 13 recommended loci in the process of identifying individuals.[3,4]

Initially, the cell-free fetal DNA is only limited to determining the type of rhesus and gender of the fetus, but currently it has grown very rapidly for the existence of specific markers for several types of trisomy in the fetus. In the field of forensic medicine it is also growing its use for invasive prenatal paternity tests. In some cases of rape that may have resulted in pregnancy, an analysis is needed to determine the birth father. Conventional prenatal paternity tests are performed by extracting chorionic villi tissue or amniotic fluid [5], but this method is invasive and poses risks to both the mother and the fetus.[6-10] The discovery of cell-free fetal DNA (cffDNA) in maternal blood provides hope for developing new prenatal paternity tests that are not in invasive and avoid the risks and complication of conventional test.[11] The isolation method of cell free fetal DNA is very important because of interference from the mother's DNA. This is the biggest challenge because the results depend on the method of isolation.[12] This study aims to detect the presence of cell free fetal DNA at 13 STR loci which can be developed for noninvasive prenatal paternity test.

METHODS

This research is a cross sectional descriptive study to detect the presence of cell-free fetal DNA at 13 Short Tandem Repeats (STR) loci and amelogenin in plasma of pregnant women. The sample of this research are 4 volunteers couple of husband and wife. Pregnant women in this study with the third trimester of pregnancy, Hb above 12 g / dl and had no complications during pregnancy such as eclampsia and others. The husband and wife agreed to take part in the study and signed the informed consent. The research approval was requested to the research ethics committee Faculty of Medicine Andalas University with number of approval 482/KEP/FK/2019.

DNA extraction

Put 20 ml pregnant women's blood into an EDTA tube. Plasma was obtained by centrifuging 20 ml of sample blood in an Eppendorf 3000 rpm tube for 10 minutes. Then centrifuged to 6000 rpm for 10 minutes, the supernatant was placed in a clean tube. Samples were stored at -200C. Extract plasma using the quick-cfDNA serum and plasma kit (Zymo Research) according to the manufacturer's instructions. Meanwhile, the blood of the mother and father was isolated using the QIAamp DNA Blood Midi Kit (Qiagen) kit.

Polymerase chain reaction (PCR)

No.	STR	PCR primers: (5 '- 3')	Thermal cycle	PCR tube
1	TPOX	F-AGAACAGGCACTTAGGGAA R-AGCGTTTATTTGCCCAA	Initial denaturation at 95^0C for 3 minutes, then followed by 35 repeated cycles, further denaturation at 95^0 C for 30 seconds, annealing at 59^0 C for 20 seconds and elongation at 72^0 C for 20 seconds. The PCR process was ended with a final elongation	The total volume of the reaction was 15 µl (7.5 µl Go Taq Green Master Mix, 2µl genomic DNA (sample), 0.5 µl Forward primers, 0.5 µl reverse primer and 4.5 µl ddH2O.
2	D3S1358	F-CTTTGGGGGCATCTCTTATAC R- AGCAAGACCCTGTCTCATAG		
3	VWA	F-LOGGATGATGATGATGATGGATAG R-AGAAGAAACAGGTCTAGAGGATCC,		
4	FGA	F-GCCCCATAGGTTTTGAACTCA R- TGATTTGTCTGTAATTGCCAGC		
5	D18S51	R-TGGTGTGTGGAGATGTCTTAC F-CTCTGAGTGACAAATTGAGACC		
6	D8S1179	F- TTTTTGTATTTCATGTGTACATTCG R-CGTAGCTATAATTAGTTCATTTTCA		
7	D21S11	F- AATATGTGAGTCAATTCCCCAAG R- AATAGGAGGTAGATAGACTGGATAG		
8	D5S818	F-CAACATTTGTATCTTTATCTGTATCC R-ATATTTTAATAGCAAGTATGTGACAAG		
9	D13S317	F- TCTGACCCATCTAACGCCTATC R- CATAGGCAGCCCAAAAAGACAG		
10	D7S820	F- ACAGAATTGCACCAAATATTG R- GGGTATGATAGAACACTTGTC		

			step at 72^0 C for 5 minutes	
11	D16S539	F-ACCACAGTTCCCATTTTTATATG R-CTATCATCCATCTCTGTTTTGTC		
12	TH01	F-CATTGGCCTGTTCCTCCTCCCTTA R-GCAGGTCACAGGGAACACAGA		
13	CSFIPO	F-AGATATTAACAGTAACTGCCTTCA R-CAGATACTATCTCCTGGTGCA		
14	AmelogeninX	F-CAGAGAGAAACTAGAGAGAGCTTTG R-TCCTCAGTTCTAGCAAACAACG		
15	Amelogenin Y	F-AGCAAGAAGTAGAAGGTGATAGG R-CAAGTTTCACCAAGGGACAAC		

The PCR amplification products were observed by using agarose gel electrophoresis 1.5% which was given GelRed DNA dye. A total of 5 µl of PCR products were electrophoresed with a voltage of 100V for 60 minutes, then agarose gel was observed under UV light using GelDoc. Furthermore, it was observed using the experion DNA 1 K Analysis Kit according to the Bio-Rad protocol.

RESULTS

The research participants were 4 volunteers pregnant women and their husbands. Pregnant women in this study were 27-33 years old with a 27-33 weeks gestational age. Tables 1, 2, 3 and 4 show the length of basepair produced in the mother, father and fetus in all samples. From the results, it can be seen that in almost every locus there is a fetal amplicon, except at locus D13S317 in all sample and at locus D5S818 in sample 4.

Table 1. Length of basepair amplicon of father, mother and fetus in couples 1

Amplicon (bp)	D3S1 38	VW A	FG A	D18S 51	D8S11 79	D21S 11	D5S8 18	D13S3 17	D7S8 20	D16S5 39	CSF1P O	TH O1	TPO X
mother	133	190	185	151	177	204	147	128	151	164	162	92	86
			192	158	193	215	155	141	159	172		97	96
Father	137	189	183	154	177	215	152	137	167	164	162	90	84
		197			197	222				172			
Fetus	129	191	185	151	176	203	147	-	148	161	159	92	84
			191	158	193	215	154		156	169		98	93

Table 2. Length of basepair amplicon of father, mother and fetus in couples 2

Amplicon (bp)	D3S1 38	VW A	FG A	D18S 51	D8S11 79	D21S 11	D5S8 18	D13S3 17	D7S8 20	D16S5 39	CSF1P O	TH O1	TPO X
mother	146	190	186	149	181	209	157	134	166	174	163	97	83
			197	155				146			183		101
Father	127	178	174	157	180	205	154	139	166	173	166	91	83
	134		181								178	97	
Fetus	150	195	189	150	188	207	157	-	165	173	167	99	85
			199	157							177		104

Table 3. Amplicons of father, mother and fetus in couples 3

Amplicon (bp)	D3S1 38	VW A	FG A	D18S 51	D8S11 79	D21S 11	D5S8 18	D13S3 17	D7S8 20	D16S5 39	CSF1P O	TH O1	TPO X
mother	140	189	185	152	177	203	151	141	151	168	155	91	82
			191		193		159		160	175	171	97	
Father	133	185	180	157	186	213	156	139	169	169	169	91	82
	141		186										
Fetus	135	177	170	140	162	188	139	-	139	154	158	92	84
			176		177		146		147	161	174	97	

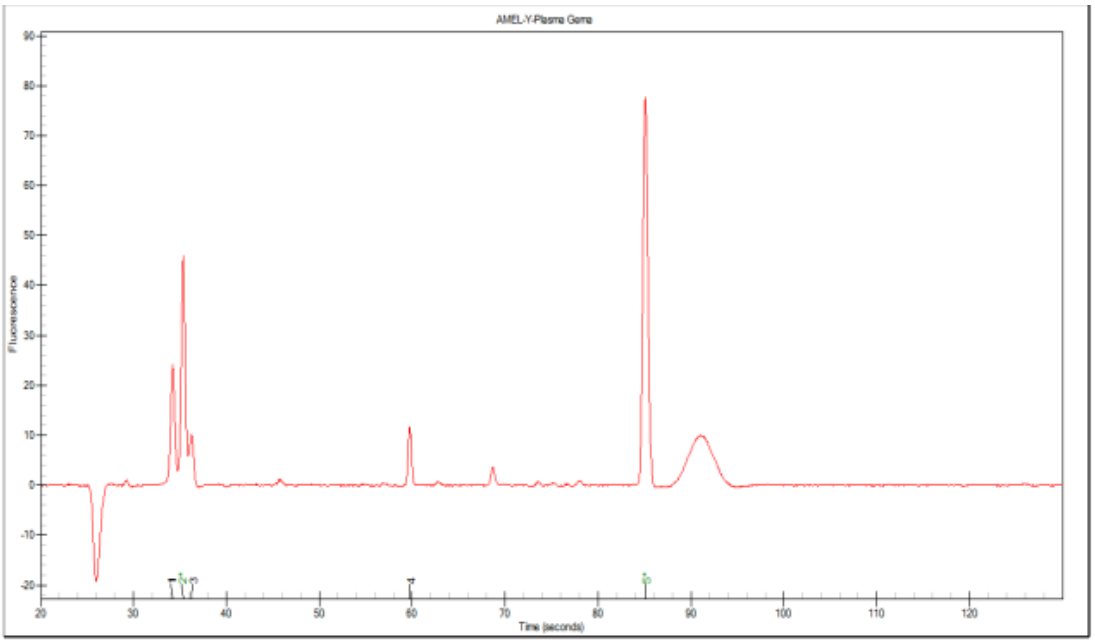
Table 4. Amplicons of father, mother and fetus in couples 4

Amplicon (bp)	D3S1358	VWA	FGA	D18S51	D8S1179	D21S11	D5S818	D13S317	D7S820	D16S539	CSF1PO	TH01	TPOX
mother	136	181 194	175 186	156	178	203	160	125 134	165	168	83	170	90
Father	145	187	185	138 145	183 190	210 220	152	138	165	170	82 92	169	88
Fetus	136	181 193	175 185	156	177	202	-	-	164	168	84	172	90

In this study we also used the Amelogenin gene, as shown in table 5 and figure 1. The sexes in cople number one and three are male and the sexes in the couple number two and four are female. In the male sex, the peak will be seen in the amelogenin Y gene, while the female gender is not found.

Table 5. Fetal sex as seen from amplicon amelogenin X and Y

Amplicon (bp)	Sample 1		Sample 2		Sample 3		Sample 4	
	X	Y	X	Y	X	Y	X	Y
cffDNA (Fetus)	163	298	164	-	150	305	164	-



Peak State	Peak Number	Mig. Time (secs)	Size (bp)	Corrected Area	Area Ratio	Concentration (ng/µl)	Molarity (nmole/l)	% Total	Observation	Comments	Peak Height
	1	34.22	0	37.80							24.35
	2	35.35	15	67.07					Lower Marker		45.91
	3	36.22	19	14.43	0.2427	1.25	97.21	76.99			10.20
	4	59.76	305	8.41	0.1415	0.37	1.85	23.01			11.68
	5	85.10	1,500	59.45					Upper Marker		77.98

Figure 1. The results of cell-free fetal DNA electrophoresis on the amelogenin Y gene.

DISCUSSION

This study aims to detect the presence of cell free fetal DNA in the plasma of pregnant women. After DNA extraction in maternal plasma, Polymerase Chain Reaction (PCR) was performed on 13 STR loci and amelogenin gen for gender detection. The use of PCR techniques in amplifying target DNA in the forensic field has been reported in various studies to have a high success rate. This is because the DNA required is relative in very small quantities.[13-15]

This study used blood samples of pregnant women from four husband and wife volunteers. Plasma for pregnant women is extracted to obtain cell free fetal DNA. Meanwhile, the DNA of the mother and father was extracted from whole blood. In tables 1,2,3 and 4 we can see the fetal amplicon at 13 STR loci. The discovery of fetal amplicons at STR loci indicates that cell free fetal DNA can be found in maternal plasma. Length of base pairs of the fetus at STR loci were found not equal to the mother, which means the amplicon different from the mother. All of the samples at locus D13S317 and one sample at locus D5S818 not detected cell free fetal cells DNA. This incident may be due to the fact that the number of cell free fetal DNA circulating in the mother's blood is small, where there is only 1 cell-free fetal DNA in 1 ml maternal plasma.[16] Besides that, the size of the fetal free cell DNA is shorter than maternal DNA.[17] Failure to identify the D13S317 and D5S818 loci can also be caused by amplification's errors or the occurrence of false negatives.on the results of the DNA multiplication process.

This study provides promising results for the use of fetal cells DNA in maternal plasma as a noninvasive prenatal paternity test specimen at the locus TH01, TPOX, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51 and D21S11 and gender checking using the amelogenin gene. Research by Agung Sosiswan which only used 5 STR loci, vWA, TH01, D13S317, D18S51, D21S11 also gave the same results. [17] The strength of this research is using 13 STR loci.

In this study we were to detect the presence of cell-free fetal DNA at almost all loci but the possibility of maternal DNA contamination remains. Compared to conventional postnatal paternity test, the greatest challenge of the cell-free fetal DNA method is the interference from maternal DNA.[18] To obtain results from cell free fetal DNA it is very important to consider the isolation method used. Several isolation methods that have been used are a column-based or magnetic beads DNA extraction kit, three enrichment methods based on size differences, regions with differential methylation (DMRs), and formaldehyde. Column-based DNA extraction method is the most widely used method for extracting cell-free fetal DNA from maternal plasma because it is easy to do, with a relatively high total DNA yield and better reproducibility.[19,20-24] This study used quick-cfDNA serum and plasma kit. This kit is column based. Several studies have shown that the Circulating Nucleic Acid and DSP virus kits are superior than the DNA Blood Mini kits. [25-27]

In Figure 1, we can see the amplicon in the amelogenin Y gene in the plasma sample of mother couple number 3 with length 305 bp. The peak in the amelogenin Y gene indicates the sex of the fetus is male. In this study, male sex was found in couple number one and three, while female fetuses were found in couple number two and four. The sex of the fetus was confirmed after the baby was born and matched 100%.

CONCLUSION

Cell-Free Fetal DNA can be detected in almost STR loci. The use of Cell-Free Fetal DNA is a potential non-invasive methods in prenatal paternity test. Additionally, the ability of the method to evaluate fetus gender has been suggested.

REFERENCES

1) Yudianto, Ahmad. Cell free fetal DNA metode non invasive dalam pemeriksaan identifikasi. Surabaya : Scopindo; 2019.
2) Hromadnikova, Extracellular nucleic acids in maternal circulation as potential biomarkers for placental insufficiency, DNA Cell Biol. 31 (2012) 1221–1232. doi:10.1089/dna.2011.1530
3) Goodwin W, Linacre A, Hadi S. An Introduction to Forensic Genetics. West Sussex: John Wiley&Sons Ltd; 2007
4) Syukriani Yoni. DNA Forensik. Bandung : Sagung Seto; 2012.
5) E.R. Norwitz, B. Levy, Noninvasive prenatal testing: the future is now, Rev. Obstet Gynecol. 6 (2013) 48–62.
6) F. Tara, M. Lotfalizadeh, S. Moeindarbari, The effect of diagnostic amniocentesis and its complications on early spontaneous abortion, Electron. Physician. 8 (2016) 2787–2792. doi:10.19082/2787.
7) M. Theodora, A. Antsaklis, P. Antsaklis, K. Blanas, G. Daskalakis, M. Sindos, S. Mesogitis, N. Papantoniou, Fetal loss following second trimester amniocentesis. Who is at greater risk? How to counsel pregnant women?, J. Matern. Fetal. Neonatal Med. 29 (2016) 590–595. doi:10.3109/14767058.2015.1012061.
8) H.V. Firth, P.A. Boyd, P. Chamberlain, I.Z. MacKenzie, R.H. Lindenbaum, S.M. Huson, Severe limb abnormalities after chorion villus sampling at 56-66 days' gestation, Lancet (London, England). 337 (1991) 762–763.
9) M. Cederholm, B. Haglund, O. Axelsson, Infant morbidity following amniocentesis and chorionic villus sampling for prenatal karyotyping, BJOG. 112 (2005) 394–402. doi:10.1111/j.1471-0528.2005.00413.x.

10) Z. Alfirevic, K. Sundberg, S. Brigham, Amniocentesis and chorionic villus sampling forprenatal diagnosis, Cochrane Database Syst. Rev. (2003) CD003252.doi:10.1002/14651858.CD003252.
11) Henningsen K. On the application of blood group testing to cases of disputed paternity in Denmark. Acta Med Leg Soc (Liege). 1956;9:95-104.
12) A.G. Vaiopoulos, K.C. Athanasoula, N Papantoniou, A. Kolialexi, Advances in Non-invasive Prenatal Diagnosis, In Vivo. 27 (2013) 165–170.
13) J.M. Butler, Genetics and genomics of core short tandem repeat loci used in human identity testing, J. Forensic Sci. 51 (2006) 253–265. doi:10.1111/j.1556-4029.2006.00046.x.
14) K. Yoshida, K. Yayama, A. Hatanaka, K. Tamaki, Efficacy of extended kinship analyses utilizing commercial STR kit in establishing personal identification, Leg. Med. (Tokyo). 13 (2011) 12–15. doi:10.1016/j.legalmed.2010.09.001.
15) K. Tamaki, R.H. Kaszynski, Q.H. Yuan, K. Yoshida, T. Okuno, T. Tsuruyama, Likelihood evaluation using 15 common short tandem repeat loci: a practical and simulated approach to establishing personal identification via sibling/parental assessments, Transfusion. 49 (2009) 578–584. doi:10.1111/j.1537-2995.2008.02024.x.
16) J. Wagner, S. Dzijan, D. Marjanovic, G. Lauc, Non-invasive prenatal paternity testing from maternal blood, Int. J. Legal Med. 123 (2009) 75–79. doi:10.1007/s00414-008-0292-9.
17) Sosiawan, A., Raharjo, D., Nuraini, I. *et al*. Detection of short tandem repeats at 5 loci and amelogenin with cell-free fetal DNA as a specimen in the development of prenatal paternity diagnostic tests. *Egypt J Forensic Sci* **8,** 15 (2018). https://doi.org/10.1186/s41935-018-0047-9
18) H. Jiang et al. Noninvasive Prenatal Paternity Testing (NIPAT) through Maternal Plasma DNA Sequencing: A Pilot Study, PLoS One. 11 (2016): e0159385. doi:10.1371/journal.pone.0159385.
19) G.B. Barra, T.H. Santa Rita, C.F. Chianca, L.F.R. Velasco, C.F. de Sousa, L.F.A. Nery, S.S.S. Costa, Fetal male lineage determination by analysis of Y-chromosome STR haplotype in maternal plasma, Forensic Sci. Int. Genet. 15 (2015) 105–110. doi:10.1016/j.fsigen.2014.11.006.
20) X. Ou, J. Gao, Y. Chen, Y. Gao, D. Qu, H. Sun, Detecting hypermethylated fetal RASSF1A sequences in maternal plasma: implications for noninvasive paternity testing in pregnancy, Transfusion. 53 (2013) 1856–1858. doi:10.1111/trf.12246.
21) J.A. Tynan, P. Mahboubi, L.L. Cagasan, D. Van Den Boom, M. Ehrich, P. Oeth, Restriction Enzyme–Mediated Enhanced Detection of Circulating Cell-Free Fetal DNA in Maternal Plasma, J Mol Diagn. 13 (2011) 382–389. doi:10.1016/j.jmoldx.2011.02.001.
22) D. Yang et al, Noninvasive fetal genotyping of paternally inherited alleles using targeted massively parallel sequencing in parentage testing cases, Transfusion. 57 (2017) 1505-1514. doi:10.1111/trf.14077.
23) A. Ryan, J. Baner, Z. Demko, M. Hill, S. Sigurjonsson, M.L. Baird, M. Rabinowitz, Informatics-based, highly accurate, noninvasive prenatal paternity testing, Genet Med. 15 (2013) 473–477. doi:10.1038/gim.2012.155.
24) T.J. Legler et al, Workshop report on the extraction of foetal DNA from maternal plasma, Prenat Diagn. 27 (2007) 824–829. doi: 10.1002/pd.1783.
25) F.B. Clausen, G.R. Krog, K. Rieneck, M.H. Dziegiel, Improvement in fetal DNA extraction from maternal plasma. Evaluation of the NucliSens Magnetic Extraction system and the QIAamp DSP Virus Kit in comparison with the QIAamp DNA Blood Mini Kit, Prenat Diagn. 27 (2007) 6–10. doi: 10.1002/pd.1605.
26) G. Repiská, T. Sedláčková, T. Szemes, P. Celec, G. Minárik, Selection of the optimal manual method of cell free fetal DNA isolation from maternal plasma, Clin Chem Lab Med. 51 (2013) 1185–1189. doi:10.1515/cclm-2012-0418.
27) A.S. Devonshire, A.S. Whale, A. Gutteridge, G. Jones, S. Cowen, C.A. Foy, J.F. Huggett, Towards standardisation of cell-free DNA measurement in plasma: controls for extraction efficiency, fragment size bias and quantification, Anal Bioanal Chem. 406 (2014) 6499–6512. doi:10.1007/s00216-014-7835-3.