Clinical Chemistry 47:9
1607–1613 (2001)

Molecular Diagnostics and Genetics

Effects of Blood-Processing Protocols on Fetal and Total DNA Quantification in Maternal Plasma

Rossa W. K. Chiu,[1] Leo L. M. Poon,[1] Tze K. Lau,[2] Tse N. Leung,[2] Eric M. C. Wong,[3] and Y. M. Dennis Lo[1*]

Background: **Recently, apoptotic cells have been found in plasma obtained by centrifugation of blood from pregnant women, raising the question of what constitutes plasma and whether plasma is truly cell free. We compared the effects of different blood-processing protocols on the quantification, DNA composition, and day-to-day fluctuation of fetal and total DNA in maternal plasma.**

Methods: **Blood samples were collected from healthy pregnant women. The blood sample from each individual was simultaneously processed by different means, including the following: Percoll separation, centrifugation, microcentrifugation, and filtration. The resulting plasma aliquots were subjected to real-time quantitative amplification of the *β-globin* (for total DNA) and *SRY* (for fetal DNA) genes. The differences in the *β-globin* and *SRY* DNA concentrations and the degree of variation between the various plasma aliquots were assessed statistically.**

Results: **Different protocols of blood processing significantly affected the quantification and the day-to-day fluctuation of total ($P < 0.001$), but not fetal (quantification, $P = 0.336$; fluctuation, $P = 0.206$), DNA in maternal plasma. The quantitative difference could be attributed to the fact that efficacies of different protocols for generating cell-free plasma vary. Processing blood samples by centrifugation followed by filtration or microcentrifugation is effective in producing cell-free plasma.**

Conclusions: **Standardization in plasma-processing protocols is needed for maternal plasma DNA analysis, especially for quantification of total DNA in maternal plasma. Such preanalytic factors may also affect other applications of plasma DNA analysis.**

Since the discovery of fetal DNA in maternal plasma and serum in 1997 *(1)*, numerous reports have confirmed its potential application for noninvasive prenatal diagnosis. The applications reported to date include the prenatal assessment of fetal gender in relation to sex-linked diseases *(1)*, fetal Rhesus D status *(2)*, and disorders of autosomal dominant inheritance, such as myotonic dystrophy *(3)* and achondroplasia *(4)*. The detection of paternally inherited microsatellite polymorphisms in maternal plasma *(5, 6)* is a promising alternative approach to the use of fetal DNA in maternal plasma for the diagnosis of paternally inherited conditions, including certain chromosomal translocations *(7)*. On the other hand, the development of quantitative assays for the detection of fetal DNA in maternal plasma *(8)* has provided another means for detection of fetal or pregnancy-associated complications by the detection of differences in fetal DNA concentrations in comparison with normal pregnancies. Such quantitative differences have been reported in preeclampsia *(9, 10)*, trisomy 21 *(11, 12)*, preterm labor *(13)*, and fetal–maternal hemorrhage *(14)*.

In addition to clinical investigations, studies have also been conducted to elucidate the biologic aspects of fetal DNA in maternal plasma. In fact, it has been shown that fetal DNA represents a substantial proportion of the total DNA in maternal plasma, contributing ~3.4% and ~6.2% of total plasma DNA in early and late pregnancy, respectively *(8)*. In addition, the concentration of fetal DNA also increases with the progression of pregnancy *(8)*. After delivery, the clearance of fetal DNA is an extremely rapid process, with a mean plasma half-life of 16.3 min *(15)*.

Despite much research being conducted in relation to fetal DNA in maternal plasma, little is known about its characteristics and biologic origin. Recently, van Wijk et al. *(16)* processed plasma by Percoll separation and reported the presence of apoptotic cells of fetal origin in plasma of pregnant women. In fact, we have reproduced

Departments of [1] Chemical Pathology and [2] Obstetrics and Gynecology, and [3] Center for Clinical Trials and Epidemiological Research, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, SAR.

*Address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Building, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong SAR. Fax 852-2194-6171; e-mail loym@cuhk.edu.hk.

Received April 12, 2001; accepted May 30, 2001.

and confirmed these findings and used fetal cells in maternal plasma obtained by Percoll separation for the noninvasive prenatal diagnosis of Down syndrome by fluorescence in situ hybridization *(17)*. These reports *(16, 17)* raise a fundamental question regarding what constitutes plasma, and whether plasma is truly acellular. Conventionally, plasma is separated from the cellular components of blood by centrifugation. Consequently, it is logical to deduce that the means of blood processing may determine what constitutes plasma. In fact, a review of the literature has revealed the use of different protocols to obtain plasma for fetal DNA assessment, including the use of different centrifugation speeds *(8, 18)* and the use of density-gradient separation *(16)*. However, there have been no reports comparing the impact of different blood-processing protocols on the concentration of fetal DNA in maternal plasma.

With the discovery of fetal cells in maternal plasma, other logical questions include whether fetal DNA circulates predominantly in a cellular or cell-free form in maternal plasma, what are the relative proportions of these forms, and whether such proportions are altered if plasma is processed by different methods. For example, Kopreski et al. *(19)* subjected sera of cancer patients to filtration, showing that a proportion of serum RNA was extracellular. Because many studies in this field have relied on the quantification of fetal DNA in maternal plasma, it would be of prime importance to investigate whether the apparent maternal plasma DNA concentration would be affected by different sample processing protocols. It would also be important to study whether such preanalytic issues would further affect the observed day-to-day fluctuation of plasma DNA concentration *(18)*.

Consequently, it is the objective of this study to investigate the effects of different blood-processing protocols on the quantitative analysis of total and fetal DNA in maternal plasma, as well as the effect on the relative proportions of cellular and cell-free DNA. The study also addresses whether the daily fluctuation in DNA concentration in maternal plasma is affected by the means from which plasma is derived. The protocols to be studied include the use of density-gradient and different centrifugation protocols.

Materials and Methods

The study comprised two modules. The first module (module 1) focused on the effects of centrifugation, microcentrifugation, and Percoll gradient separation on the concentrations of total and fetal DNA, as well as cellular and cell-free DNA in maternal plasma. To study the relative proportion of cell-free DNA, aliquots of plasma were subjected to filtration by a 0.2-μm filter to remove the cells that might have remained in plasma after initial processing. The difference in the DNA concentration between filtered and nonfiltered plasma reflected the proportion of DNA derived from intact cells in plasma. The protocol used by our laboratory was evaluated among the other protocols. Our current protocol involves initial centrifugation of blood samples at 1600*g* (Megafuge 1.0R; Heraeus Instruments) for 10 min. The plasma is then transferred into polypropylene tubes, with care not to disturb the buffy coat layer, and the plasma is further subjected to microcentrifugation for 10 min, at full speed (16 000*g*; Eppendorf Centrifuge 5415D). This recentrifuged plasma is then harvested for DNA extraction followed by PCR analysis.

The second module (module 2) was designed primarily to assess whether differences in plasma DNA concentration because of blood processing by different protocols would affect the degree of daily fluctuation of maternal plasma DNA. Recently, Zhong et al. *(18)* reported that total DNA in maternal plasma fluctuated by an average of 13.5-fold. They based their observation on analysis of plasma obtained by one-step centrifugation at 800*g* for 10 min. Consequently, to test our hypothesis, three protocols of plasma processing, including 800*g* centrifugation, filtration, and our current laboratory protocol, were selected to assess the effects on plasma DNA quantification, and, furthermore, whether such effects would bias the interpretation of the degree of daily fluctuation.

All plasma aliquots in both modules were subjected to PCR amplification of both the *β-globin* and *SRY* genes *(15)*, which were chosen to reflect the concentration of total DNA and fetal DNA in maternal plasma, respectively.

pregnant women

Normal pregnant women bearing a male fetus and attending the Department of Obstetrics and Gynecology at the Prince of Wales Hospital, Hong Kong, were recruited with informed consent. Ethical approval was obtained from the Clinical Research Ethical Committee of The Chinese University of Hong Kong. The fetal gender was ascertained by ultrasound scan. Antecubital venous blood samples (10–12 mL) were collected into EDTA tubes. The blood samples were processed within 2 h of sample collection. The plasma was transferred into polypropylene tubes and stored at −20 °C until further processing.

processing of blood samples

Module 1. EDTA blood (10 mL) was collected from each individual. Specimen (6 mL) was centrifuged at 1600*g* for 10 min (Megafuge 1.0R; Heraeus Instruments). The supernatant was transferred into polypropylene tubes, with particular attention not to disturb the buffy coat layer. The plasma was then subjected to four different treatments. One-fourth of the plasma was subjected to no additional treatment, and one-fourth was subjected to filtration by a 0.2-μm filter. The remainder was microcentrifuged (Eppendorf Centrifuge 5415D) at full speed (16 000*g*), one-half of which was subjected to further filtration.

The remaining 4 mL of blood from each individual was diluted with 2 mL of phosphate-buffered saline, and

fractionated by centrifugation (1200*g*) on a discontinuous Percoll (Amersham Pharmacia) gradient (60%, 55%, 50%, 45%, and 40% in phosphate-buffered saline) *(16, 17)*. The top layer was carefully transferred to polypropylene tubes with care not to disturb the underlying column. One-third of the plasma derived by Percoll separation was filtered, another one-third was subjected to microcentrifugation at full speed (16 000*g*), and the remainder was subjected to no further treatment.

Module 2. EDTA blood (12 mL) was collected from each individual, and 8 mL was centrifuged at 800*g* for 10 min. The supernatant was carefully transferred into polypropylene tubes. One-half of the plasma was also filtered, and the remaining one-half was stored directly until processing. The remaining 4 mL of blood sample from each individual was processed by our current laboratory protocol. For women who further consented to serial blood sampling, blood samples were collected at approximately the same time of the day (between 0900 to 1100) over 3 consecutive days.

DNA EXTRACTION

DNA extraction from the differentially processed plasma aliquots was performed using a QIAamp Blood Kit (Qiagen) by use of the blood and body fluid protocol according to the manufacturer's recommendations. Plasma was used for DNA extraction (800 μL per column).

REAL-TIME QUANTITATIVE PCR

All of the plasma aliquots were subjected to real-time quantitative PCR amplification for both the *SRY* and *β-globin* genes as described previously *(8)*. Extracted plasma DNA (5 μL) was used for amplification. Real-time quantitative PCR was performed by use of an Applied Biosystems 7700 Sequence Detector (Applied Biosystems). The theoretical and practical aspects of real-time quantitative PCR have been described in detail elsewhere *(20)*. Duplicate analysis was performed for each sample, and the mean result was used for further analysis. A calibration curve was analyzed in parallel with each assay. The results were expressed as genome-equivalents by use of the conversion factor of 6.6 pg of DNA per cell *(15)*. Amplification data were analyzed and stored by the Sequence Detection System Software (Ver. 1.6.3; Applied Biosystems). The plasma DNA concentration expressed in genome-equivalents per milliliter was calculated as described previously *(8)*. Dilution of plasma samples by phosphate-buffered saline was taken into account in the calculation for plasma aliquots subjected to Percoll separation by multiplication with the respective dilution factor.

STATISTICAL ANALYSIS

Data analysis was performed by use of Sigma Stat 2.0 (SPSS).

Results

SUBJECTS

Thirty-four subjects were recruited for module 1. The gestational age was 13–38 weeks. Seventeen subjects, 9 of whom consented for serial blood sampling, were recruited for module 2, and the gestational age was 17–40 weeks.

MODULE 1

The quantitative relationships between the *β-globin* and *SRY* concentrations for the differentially processed plasma aliquots are shown in Figs. 1 and 2. Data shown in the top panel of Fig. 1 reveal that plasma processed by

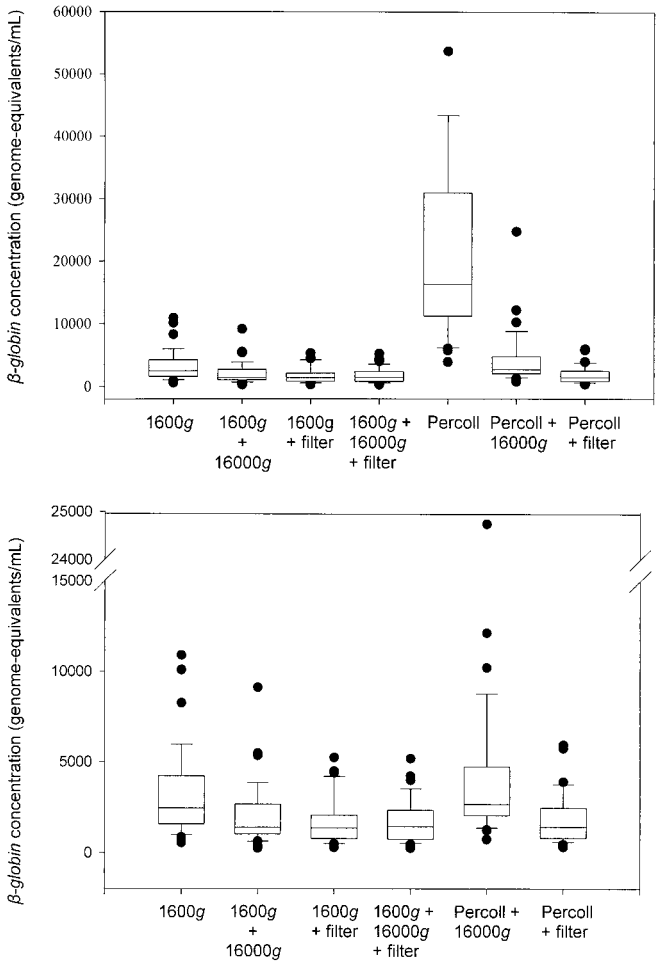


Fig. 1. Box plots of *β-globin* concentrations of the plasma aliquots prepared by different protocols.

(*Top*), box plot of *β-globin* concentrations of the plasma aliquots prepared by different protocols (as indicated on the *y axis*) for module 1. *1600*g, the speed of centrifugation; *16 000*g, the speed of microcentrifugation; *filter*, the process of filtration; *Percoll*, process of Percoll density-gradient separation. The *upper and lower limits of the boxes* and the *line across the boxes* indicate the 75th and 25th percentiles and the median, respectively. The *upper and lower horizontal bars* indicate the 90th and 10th percentiles, respectively. Outliers are illustrated as ●. (Statistics are presented in the same format for all of the box plots in this report.) (*Bottom*), box plot of *β-globin* concentration of the same treatment groups as indicated in the *top panel*.) Data from the *Percoll* group are omitted to clearly illustrate the quantitative relationship between the other treatment groups.

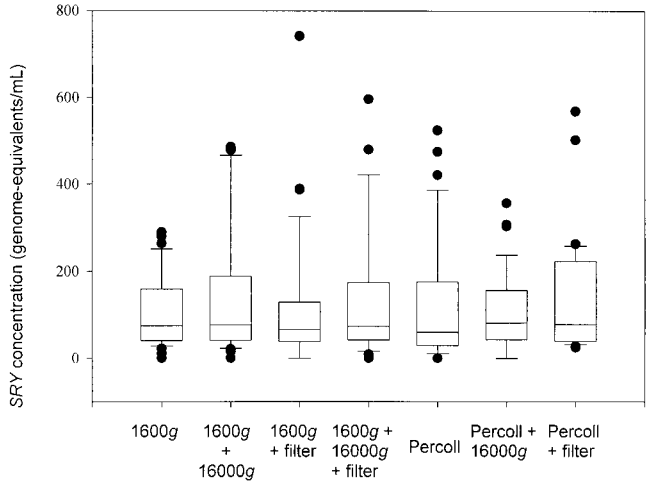


Fig. 2. Box plot of *SRY* concentration of the plasma aliquots prepared by different protocols for module 1.

Data are presented in the same format as Fig. 1.

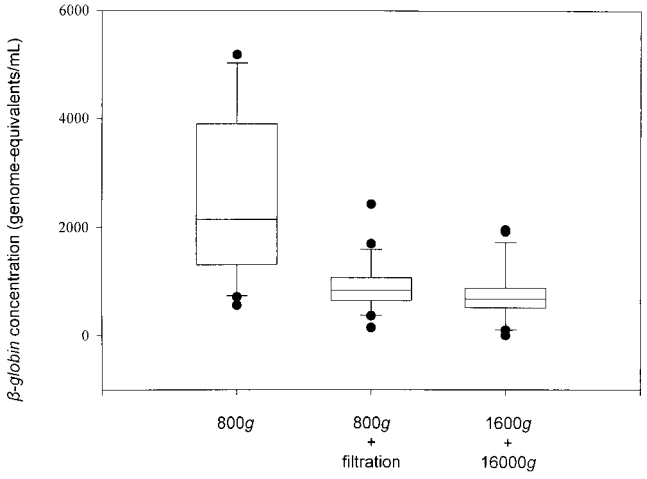


Fig. 3. Box plot of *β-globin* concentration of plasma aliquots prepared by protocols (as indicated on the *y axis*) for module 2.

*800*g and *1600*g denote the speeds of centrifugation, and *16 000*g denotes the speed of microcentrifugation.

Percoll separation led to markedly increased *β-globin* concentrations compared with the other methods. In the bottom panel of Fig. 1, the same data as in the top panel are presented, except that data from the group processed solely by Percoll separation have been omitted; so that the quantitative relationship between the other groups can be clearly assessed. The corresponding data for the *SRY* concentrations in the differentially processed plasma aliquots are illustrated in Fig. 2.

The difference in DNA concentrations between the differentially treated plasma aliquots was tested for statistical significance by Friedman repeated-measures analysis of variance on ranks. The difference for the *SRY* data among the different treatment groups did not reach statistical significance ($P = 0.336$), whereas a statistically significant difference was shown for the *β-globin* data ($P < 0.001$). As a result, to isolate the groups that differ significantly from other groups, pairwise multiple comparison by the Dunnett method was performed for the *β-globin* data. To perform the Dunnett method, a control group needed to be selected for comparison with the other groups. Centrifugation at 1600*g* with subsequent microcentrifugation and filtration was assigned as the control treatment group because this protocol involved both filtration and centrifugation at the highest speeds, and thus would be expected to remove virtually all cellular elements in plasma.

Results of the Dunnett test revealed that *β-globin* concentrations from plasma aliquots of the three treatment groups involving Percoll separation with or without further microcentrifugation and centrifugation at 1600*g* alone were significantly higher than in the control group ($P < 0.05$). On the other hand, the *β-globin* concentrations of the remaining treatment groups were not significantly different from those in the control group.

MODULE 2

Data analysis for module 2 was divided into two phases: (*a*) the data were analyzed to determine whether the different treatment groups studied (centrifugation at 800*g*, with or without filtration, and our current laboratory protocol) led to a statistically significant difference in *β-globin* and *SRY* concentrations; (*b*) when such difference was demonstrated, the degree of fluctuation in serial *β-globin* and *SRY* concentrations from each woman was then analyzed.

The first phase analysis involved blood samples taken on one occasion from 17 subjects. The *β-globin* and *SRY* concentrations of the differentially treated plasma aliquots are shown in Figs. 3 and 4, where the *β-globin* or *SRY* concentrations for the three treatment groups are presented by box plots. The data among the different treatment groups were tested by Friedman repeated-

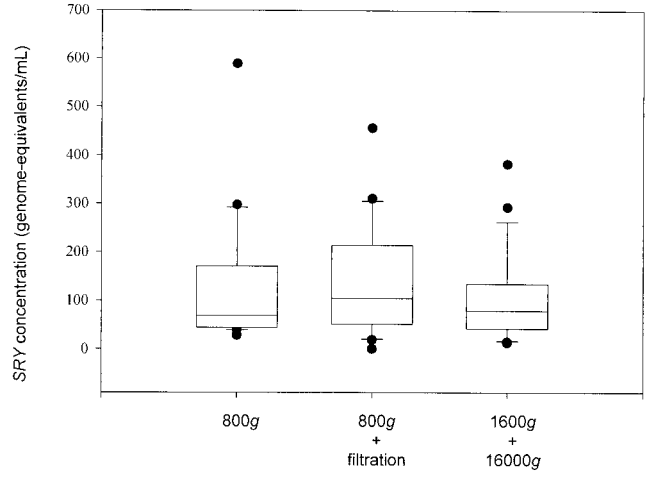
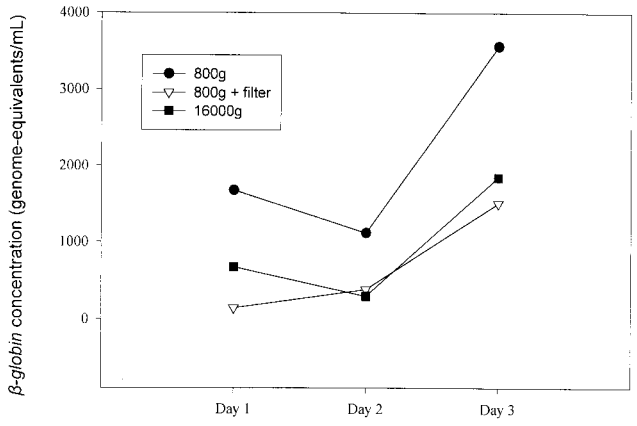


Fig. 4. Box plot of *SRY* concentration for module 2 presented in the same format as Fig. 3.



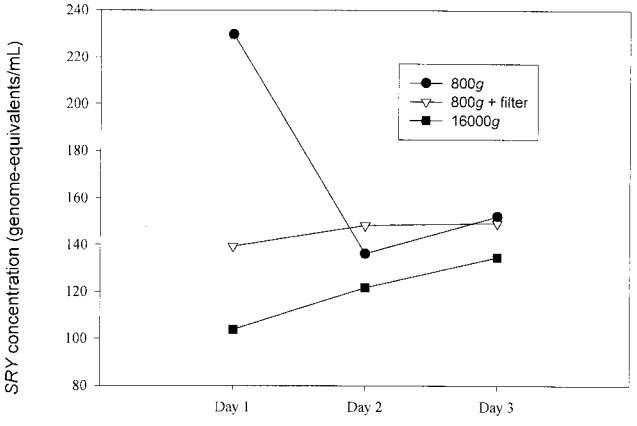


Fig. 5. Serial *β-globin* and *SRY* concentrations of sample 331 are illustrated in the *top* and *bottom panels*, respectively.

measures analysis of variance on ranks and the Dunnett method as described in module 1. No statistical significance was shown for the *SRY* data ($P = 0.204$), whereas a statistically significant difference was observed among the different treatment groups with regard to *β-globin* concentrations ($P < 0.001$). The Dunnett pairwise multiple comparison was performed subsequently. On the basis of the data of module 1, which revealed that plasma processed by filtration led to the lowest DNA concentrations, filtered plasma was selected as the control treatment group. Compared with the control group, *β-globin* concentrations in the plasma samples processed by 800*g* centrifugation alone were significantly higher ($P < 0.05$), whereas no difference was seen with the microcentrifuged plasma.

Among the 17 individuals, 9 women participated in further blood sampling for 2 more consecutive days (3 days in total). The samples were processed and analyzed in the same manner for 3 days. The 3-day *β-globin* and *SRY* concentrations of the different plasma aliquots de-

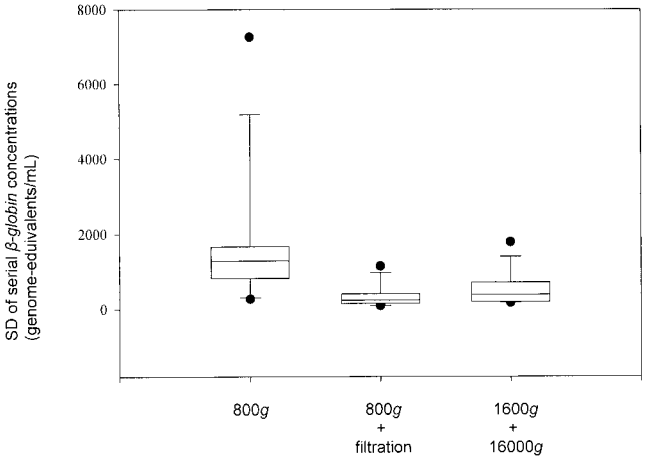


Fig. 6. Box plot of the SD of the serial *β-globin* concentrations for the three treatment groups (as indicated on the *y axis*).

rived from the blood sample of one of the individuals are shown in Fig. 5. To assess the degree of fluctuation in DNA concentration over 3 days, the mean and SD of the 3-day DNA values for each modality of plasma processing, were calculated. It was expected that large fluctuations in the DNA concentration would lead to a high SD and vice versa. The SD values were used to compare the degree of fluctuation among the three treatment groups. The SDs for the 3-day *β-globin* and *SRY* concentrations resulting from the three different treatment protocols are shown in Figs. 6 and 7. To assess the significance in the differences in the SD values, Friedman repeated-measures variance on ranks was performed. The difference in the SD values for the *SRY* data did not show any statistical significance among the groups ($P = 0.206$). However, the SD values for the *β-globin* data were significantly different among the groups ($P < 0.001$). In the filtration group as control for Dunnett comparison, the SD values for 800*g* centrifugation alone were significantly higher ($P < 0.05$),

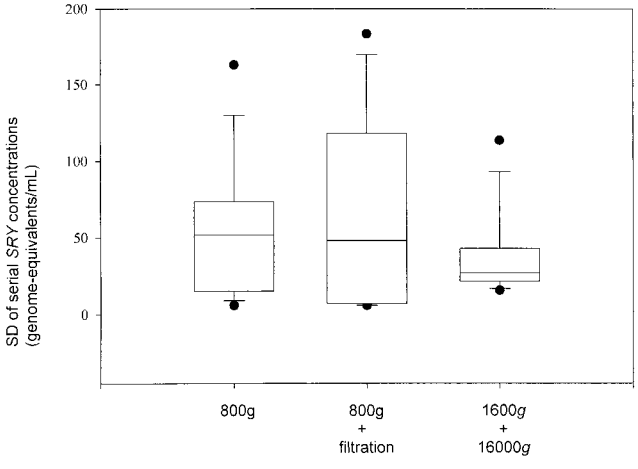


Fig. 7. Box plot of the SD of the serial *SRY* concentrations for the three treatment groups (as indicated in the *y axis*).

whereas no significant difference was observed when these values were compared with those of the microcentrifuged plasma.

Discussion

Analysis of data from both modules consistently revealed that different blood-processing protocols have a significant impact on the quantification of *β-globin*, but not *SRY* sequences in plasma. *β-globin* is representative of the total DNA (both maternal and fetal), whereas *SRY* is representative of fetal DNA in maternal plasma of women bearing a male fetus. In other words, by altering the blood-processing protocol, quantification of total, but not fetal, DNA is affected. Before suggesting a potential explanation for this observation, one must first reconsider the question of what constitutes plasma. Plasma aliquots subjected to filtration consistently demonstrate the lowest total DNA concentrations. Plasma filtration by a submicron filter is used to remove residual cells that remain in plasma after the initial centrifugation step. In other words, the DNA concentration in filtered plasma reflects the proportion of "extracellular" fetal and total DNA in the blood sample. Furthermore, the DNA concentration in filtered plasma aliquots could be used as a baseline value to compare the effectiveness of the various blood-processing protocols in producing cell-free plasma. Because plasma subjected to microcentrifugation (except when pretreated by Percoll separation) consistently leads to a total DNA concentration that is statistically similar to that of filtered plasma, we infer that microcentrifugation is just as effective at generating cell-free plasma as filtration.

On the contrary, centrifugation alone, by various speeds (1600*g* and 800*g*) led to total DNA concentrations that were significantly different and higher than those of filtered plasma ($P < 0.05$). Therefore, it can be deduced that despite centrifugation, some of the maternal cells could remain in plasma, leading to an increase in the total DNA in plasma. In addition, the total DNA concentration of plasma aliquots processed by Percoll separation was strikingly different and higher than that of filtered plasma ($P < 0.05$). Consequently, it can be inferred that centrifugation with a Percoll gradient is very ineffective in removing cells from plasma and possibly enriches for cellular components in the resulting plasma. The cellular proportion in plasma is so high that even additional microcentrifugation of plasma initially processed by Percoll separation leads to a total DNA concentration that is significantly higher than filtered plasma. It is interesting to note that both recent reports *(16, 17)* of fetal cell detection in maternal plasma used Percoll density-gradient separation, a result that is consistent with our current observation that Percoll columns substantially increase the proportion of cells in plasma and enhance the detection of fetal cells.

Having considered that altering the modality of sample processing can cause changes in the proportion of cellular and extracellular plasma DNA, one can return to seek a potential explanation for the differential effect of the various protocols on the quantification of total and fetal DNA. The lack of difference in fetal DNA concentration among the different treatment groups, including Percoll gradient separation, compared with the filtered plasma aliquots suggests that most of the fetal DNA circulates in an extracellular form. It is known that in a background of 300–400 nucleated cells/mL of Percoll-derived maternal plasma *(16)*, fetal cells are detectable at a frequency of up to 0.4% *(17)*. This amounts to the detection of ~2 fetal cells/mL of Percoll-derived maternal plasma. From our data, plasma aliquots processed by Percoll gradient contain a mean of 124 genome-equivalents/mL of fetal DNA. Consequently, intact fetal cells contribute only a very small proportion of the quantifiable fetal DNA. Hence, quantification of fetal DNA is not affected despite the varied efficiency of different protocols in generating cell-free plasma.

The implication of these findings on the previously published data involving the quantification of fetal DNA in maternal plasma is that observations made from the use of fetal DNA concentrations alone remained valid, no matter which protocol was used. However, until the means from which we derive plasma is standardized, observations or conclusions drawn from expression of results as ratios of fetal to total DNA should be discouraged.

Data from module 2 reveal that the use of different blood-processing protocols affects not only the quantification of total DNA in maternal plasma, but also the degree of day-to-day variation in total DNA concentration. In contrast, the day-to-day variation of fetal DNA concentrations is not affected. The implication of this observation is that assessments of the biologic variation of maternal DNA in plasma can be biased by the way blood samples are processed *(18)*. There is no significant difference between the degree of fluctuation in the total DNA concentration of plasma aliquots processed by modalities (filtered or microcentrifuged) that are effective in producing cell-free plasma. On the contrary, the degree of fluctuation is different and greater in plasma subjected to 800*g* centrifugation alone. A potential explanation for the greater degree of fluctuation in plasma processed by centrifugation compared with cell-free plasma is that centrifugation alone is not effective in removing all of the cells in plasma, and the number of cells that remain in plasma after processing is variable. This variation markedly affects the day-to-day variation of quantification of total plasma DNA concentration. Assuming that true biologic variation is constant over time, the increased imprecision leads to an increased degree of fluctuation as observed. On the other hand, as fetal DNA in maternal plasma mainly exists in extracellular form, despite the inefficiency of certain protocols to produce cell-free plasma, the degree of fluctuation is not significantly affected.

This study has provided evidence that both maternal

and fetal DNA do circulate in extracellular form in maternal plasma. Different protocols of blood sample processing impart a significant effect on the quantification of total DNA in maternal plasma. Virtually cell-free plasma can be obtained by centrifugation of blood samples, followed by filtration or microcentrifugation. We recommend the use of the latter approach because it is both simpler and less costly to implement than filtration. The current findings may be applicable to other fields of research on circulating nucleic acids, including the use of plasma DNA in cancer diagnosis *(21, 22)*, detection of transplant rejection *(23)*, trauma assessment *(24)*, and the growing field of plasma RNA research *(19)*. For example, for the quantitative measurement of epigenetic tumor markers in plasma, several investigators have proposed the use of the ratio of methylated to nonmethylated or total circulating DNA in plasma *(25–27)*. By highlighting the importance of centrifugation protocols for plasma processing, our data have obvious bearing on this type of analysis. As research in the field of circulating nucleic acids is growing rapidly for findings to be easily comparable across studies, some form of standardization needs to be agreed on.

The work is supported by the Innovation and Technology Fund (AF/90/99) and the Hong Kong Research Grants Council (CUHK 4255/99 M). We thank Dr. Zhang Jun for technical help.

References

1. Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.
2. Lo YMD, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 1998;339:1734–8.
3. Amicucci P, Gennarelli M, Novelli G, Dallapiccola B. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. Clin Chem 2000;46:301–2.
4. Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihara T. Prenatal DNA diagnosis of a single-gene disorder from maternal plasma [Letter]. Lancet 2000;356:1170.
5. Tang NL, Leung TN, Zhang J, Lau TK, Lo YMD. Detection of fetal-derived paternally inherited X-chromosome polymorphisms in maternal plasma. Clin Chem 1999;45:2033–5.
6. Pertl B, Sekizawa A, Samura O, Orescovic I, Rahaim PT, Bianchi DW. Detection of male and female fetal DNA in maternal plasma by multiplex fluorescent polymerase chain reaction amplification of short tandem repeats. Hum Genet 2000;106:45–9.
7. Chen CP, Chern SR, Wang W. Fetal DNA in maternal plasma: the prenatal detection of a paternally inherited fetal aneuploidy. Prenat Diagn 2000;20:355–7.
8. Lo YMD, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768–75.
9. Lo YMD, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. Clin Chem 1999;45:184–8.
10. Zhong XY, Laivuori H, Livingston JC, Ylikorkala O, Sibai BM, Holzgreve W, et al. Elevation of both maternal and fetal extracellular circulating deoxyribonucleic acid concentrations in the plasma of pregnant women with preeclampsia. Am J Obstet Gynecol 2001;184:414–9.
11. Lo YMD, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, et al. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. Clin Chem 1999;45:1747–51.
12. Zhong XY, Burk MR, Troeger C, Jackson LR, Holzgreve W, Hahn S. Fetal DNA in maternal plasma is elevated in pregnancies with aneuploid fetuses. Prenat Diagn 2000;20:795–8.
13. Leung TN, Zhang J, Lau TK, Hjelm NM, Lo YMD. Maternal plasma fetal DNA as a marker for preterm labour. Lancet 1998;352:1904–5.
14. Lau TK, Lo KW, Chan LY, Leung TY, Lo YMD. Cell-free fetal deoxyribonucleic acid in maternal circulation as a marker of fetal-maternal hemorrhage in patients undergoing external cephalic version near term. Am J Obstet Gynecol 2000;183:712–6.
15. Lo YMD, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999;64:218–24.
16. van Wijk IJ, de Hoon AC, Jurhawan R, Tjoa ML, Griffioen S, Mulders MA, et al. Detection of apoptotic fetal cells in plasma of pregnant women. Clin Chem 2000;46:729–31.
17. Poon LLM, Leung TN, Lau TK, Lo YMD. Prenatal detection of fetal Down's syndrome from maternal plasma. Lancet 2000;356:1819–20.
18. Zhong XY, Burk MR, Troeger C, Kang A, Holzgreve W, Hahn S. Fluctuation of maternal and fetal free extracellular circulatory DNA in maternal plasma. Obstet Gynecol 2000;96:991–6.
19. Kopreski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in the serum of patients with malignant melanoma. Clin Cancer Res 1999;5:1961–5.
20. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996;6:986–94.
21. Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2:1033–5.
22. Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 1996;2:1035–7.
23. Lo YMD, Tein MS, Pang CC, Yeung CK, Tong KL, Hjelm NM. Presence of donor-specific DNA in plasma of kidney and liver-transplant recipients [Letter]. Lancet 1998;351:1329–30.
24. Lo YMD, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. Clin Chem 2000;46:319–23.
25. Lo YMD, Wong IH, Zhang J, Tein MS, Ng MH, Hjelm NM. Quantitative analysis of aberrant p16 methylation using real-time quantitative methylation-specific polymerase chain reaction. Cancer Res 1999;59:3899–903.
26. Kawakami K, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, et al. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. J Natl Cancer Inst 2000;92:1805–11.
27. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001;61:1659–65.