Prenatal screening in twin pregnancies

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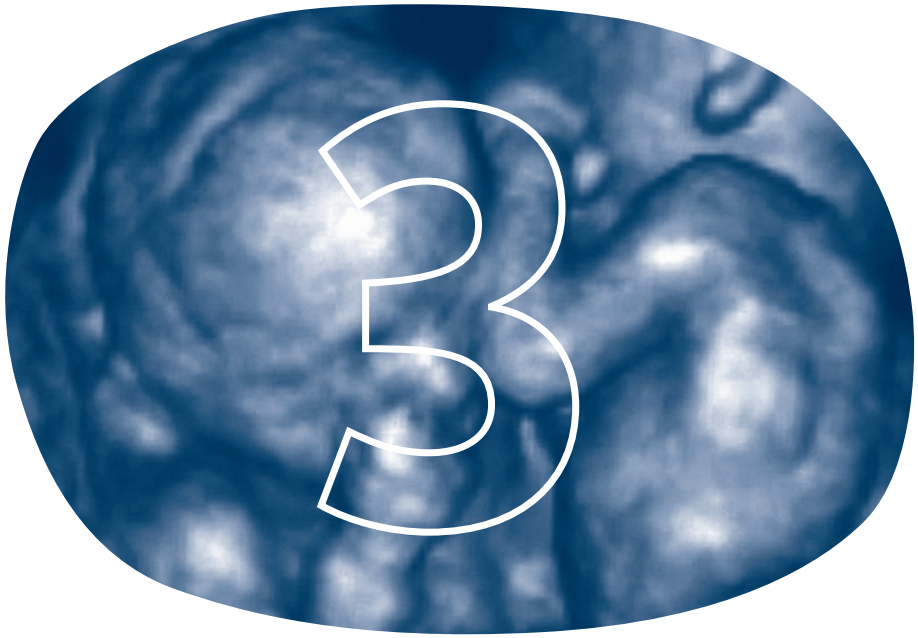
22) Wojdemann KR, Larsen SO, Shalmi AC, Sundberg K, Tabor A, Christiansen M. Nuchal translucency measurements are highly correlated in both mono- and dichorionic twin pairs. Prenat Diagn 2006 Mar;26(3): 218-20.
23) Johnson MR, Abbas A, Nicolaides KH. Maternal plasma levels of human chorionic gonadotrophin, oestradiol and progesterone in multifetal pregnancies before and after fetal reduction. J Endocrinol 1994 Nov;143(2):309-12.
24) Muller F, Dreux S, Dupoizat H, Uzan S, Dubin MF, Oury JF, et al. Second-trimester Down syndrome maternal serum screening in twin pregnancies: impact of chorionicity. Prenat Diagn 2003 Apr;23(4):331-5.
25) Chasen ST, Perni SC, Kalish RB, Chervenak FA. First-trimester risk assessment for trisomies 21 and 18 in twin pregnancy. Am J Obstet Gynecol 2007 Oct;197(4):374-3.



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Processing effects and storage conditions on A Disintegrin and Metalloprotease (ADAM12s), a maternal serum marker for adverse pregnancy outcome

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Letter to the editor

Prenatal screening for chromosomal abnormalities, especially trisomy 21 (Down syndrome) has become a part of routine obstetric care. Currently in the Netherlands, testing in the first trimester using the combined test is the test of choice [1]. This combined test calculates the individual woman's risk for a Down syndrome affected pregnancy based on maternal age, maternal serum levels of free β- human chorionic gonadotrophin (free β-hCG) and pregnancy associated plasma protein-A (PAPPA), along with ultrasound measurement of the fetal nuchal translucency. Maternal serum is collected between 9 and 14 weeks of pregnancy. Women with a screen-positive test result (cut-off 1:200) are offered an invasive diagnostic test, amniocentesis or chorionic villus biopsy, to determine fetal chromosomes. Invasive tests are associated with an iatrogenic risk of miscarriage. Recently the potential value of 'A Disintegrin and Metalloprotease 12s' (ADAM12s) has been described as an indicator of fetal abnormalities. ADAM12s is the short and secreted splice form of ADAM12 and a placenta derived glycoprotein produced by trophoblasts [2]. ADAM12s, like PAPP-A, is an insulin-like growth factor protease that has a role in regulating fetal growth by controlling the amount of bio available insulin-growth factor (IGF) I and II. Low PAPP-A and ADAM12s levels have been associated with an increase of insulin-like growth factor binding protein (IGFBP)-3 and -5 resulting in low free IGF concentrations. Previous studies showed reduced ADAM12s levels in first trimester maternal serum in trisomy affected pregnancies [3-5]. Adding ADAM12s to the current first combined test could result in a higher detection rate and lower false positive rate. Moreover, first trimester ADAM12s levels have been reported to be reduced in pregnancies complicated by preeclampsia or fetal growth restriction [6-8].
In this letter, we describe the results of experiments that investigated the effects of processing and formulated guidelines for optimal storage of ADAM12s. In these experiments, the stability of ADAM12s in serum samples stored at -20°C, 4°C, 18°C and 37°C up to 25 days was investigated. In addition the effect of repeated freeze/thaw cycles on ADAM12s concentrations and effect of centrifugation were studied. Finally, in addition to investigating the stability of ADAM12s in serum, we also studied some basic characteristics of the ADAM12s assay. This included intra- and inter-assay variation, lot-to-lot variation, linearity, and matrix interference. Pregnant women with gestational ages between 10 and 32 weeks, participating in the study gave informed consent. ADAM12s concentrations were quantitated using a solid phase, two-site fluoroimmunometric assay based on a direct sandwich technique with the AutoDelfia analyzer (PerkinElmer, Turku, Finland). Regression analysis was performed according to Passing and Bablok, MedCalc 7.4 software (Mariakerke, Belgium) [9]. The intra-assay coefficient of variation (CV) was calculated from the difference between duplicate measurements. Minimal variation, < 1.5% at all levels, was seen using 78 serum samples measured in duplicate. Inter-assay CV for three commercially available pools (PerkinElmer) and three human serum pools at different levels was <3.5% (n=16). Linearity was good and the mean recovery from two samples diluted 1:1 with the DELFIA Diluent II for ADAM12s was added, was 101% (range 97%-105%). To evaluate lot-to-lot variation, 144 serum samples were determined on the same day with two different kit lot numbers (422590 and 459296). Passing and Bablok analysis demonstrated that the agreement between the two lot numbers was excellent (r >0.99, p<0.01) with a slope of 1.076 and intercept of -16.82. To evaluate equality of measurements between two different AutoDelfia's 24 samples were analyzed.
The agreement for ADAM12s analysis was excellent between the AutoDelfia instruments according to Passing and Bablok analysis (r>0.99, p<0.01) with a slope of 1.01 and intercept of 7.15. To evaluate matrix interference, we analyzed serum from five subjects collected into three different tubes: a plastic tube with gel and clot activator, a glass tube with clot activator but without gel, and a tube with gel and lithium heparin (BD Diagnostics, Franklin Lakes, NJ, USA). According to the PerkinElmer ADAM12s kit insert, EDTA and citrate influence ADAM12s measurements, and were thus excluded from analysis. In all three tube types that were tested, ADAM12s detection was possible and results were comparable, i.e. a factor 0.98 for the tubes with gel and lithium heparin and a factor 0.99 for tubes with gel and clot activator compared with serum from glass tubes with clot activator. To study the stability of ADAM12s relative concentrations of ADAM12s in serum from four patients, assayed on the same day, were measured in tubes stored at -20°C, 4°C, 18°C and 37°C with the baseline value -80°C– set at 100%. Figure 1 shows the mean percentages for ADAM12s from samples from four patients stored at -20°C, 4°C, 18°C and 37°C against time. The ADAM12s concentration was highest at baseline -80°C and was stable when stored at -20°C for at least 25 days and at 4°C for at least 4 days. Figure 2 shows the individual absolute ADAM12s concentrations in serum samples of six patients stored at -80°C versus the number of freeze/thaw cycles. We observed no change in ADAM12s concentrations during six freeze/thaw cycles. Figure 3 demonstrates that samples stored at 4°C and centrifuged after 1, 4, 48, 72, 96 h showed no differences in ADAM12s concentrations. ADAM12s stored at 4°C remained stable over 96 h following collection.
In conclusion, we showed that ADAM12s is stable when stored at -20°C for at least 25 days and at 4°C for at least 4 days. Concentrations are not influenced by multiple freeze/thaw cycles and the blood samples can be stored for 96 h at 4°C prior to centrifugation. This considerable stability allows a wide spread application of ADAM12s measurement in obstetric practice.

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Figure 1 Relative percentages of ADAM12s in serum samples stored at -20°C, 4°C, 18°C and 37°C

Mean (± SD error bars) percentages of ADAM12s plotted vs. time, serum samples collected from four subjects (gestational age ranged from 15 to 29 weeks) and stored at -20°C, 4°C, 18°C, and 37°C, compared with baseline sample stored at –80°C and assumed to represent 100%. ADAM12s ranged from 1080 up to 4992 ng/mL

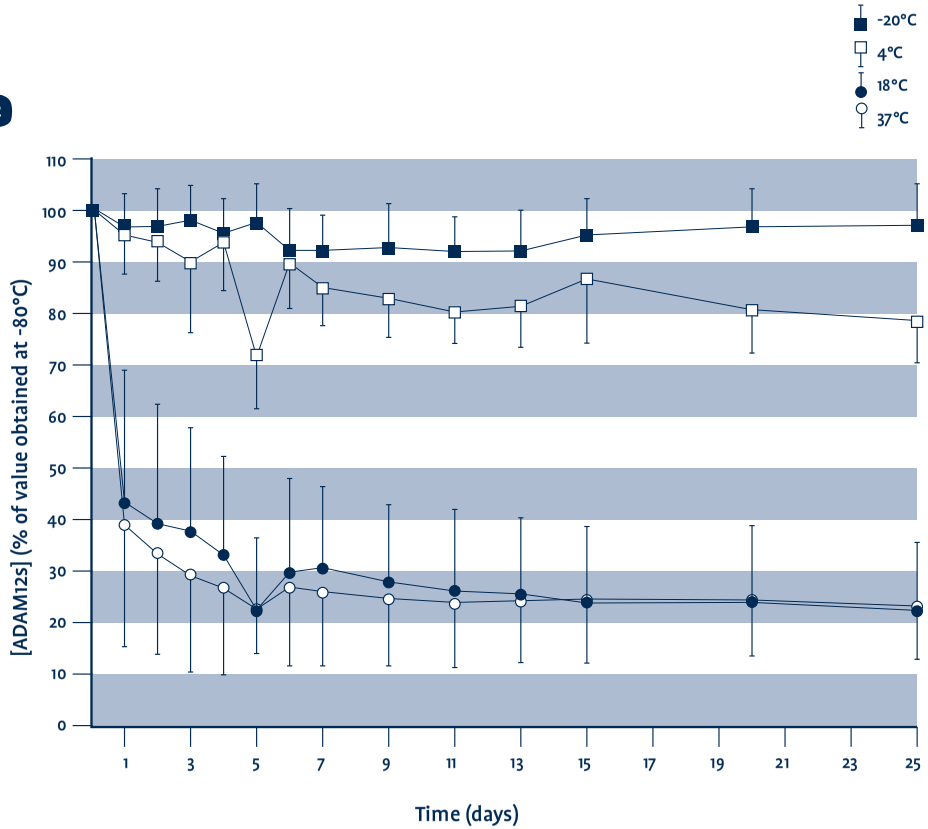


Figure 2 Effect of repeated freezing and thawing of serum on ADAM12s concentration

Individual absolute ADAM12s concentrations in samples from six subjects (gestational age ranged from 10-32 weeks) versus the number of freeze/thaw cycles

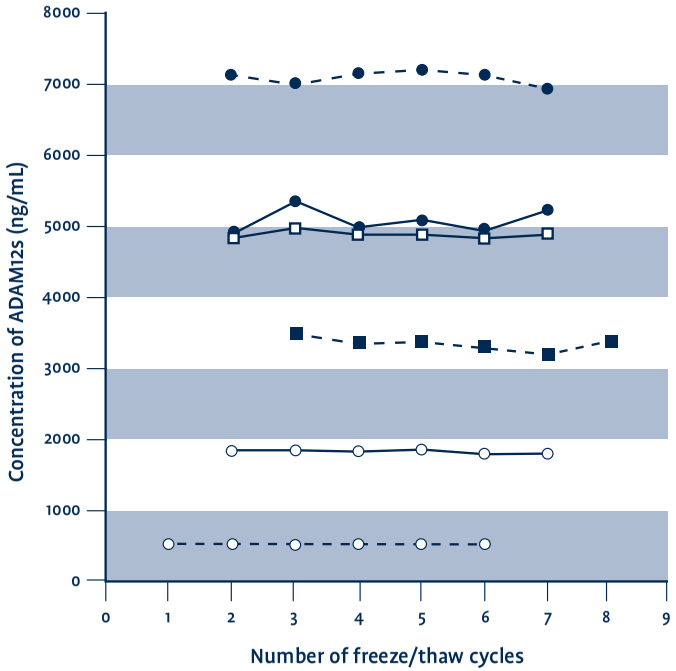
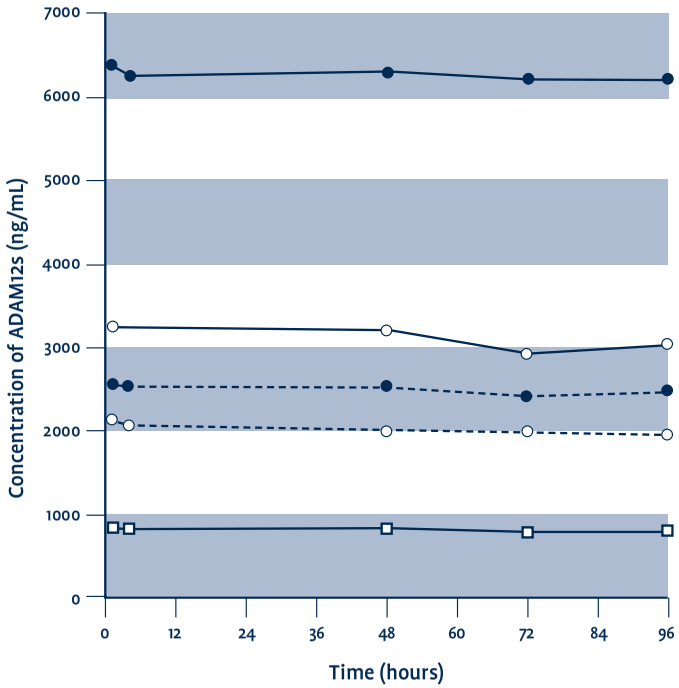


Figure 3 Influence of time to centrifugation on ADAM12s concentration

Individual absolute ADAM12s concentrations in serum samples from five subjects (gestational age ranged from 15 to 22 weeks) that were stored at 4°C and centrifuged after 1, 4, 48, 72 and 96 h following collection



References

1) Go AT, Hupkes HW, Lomecky M, Twisk J, Blankenstein MA, Van Vugt JM. [Evaluation of a programme for the prenatal screening for Down's syndrome by ultrasonographic nuchal translucency measurement and serum determinations in the first trimester of pregnancy]. Ned Tijdschr Geneeskd 2005 Dec 10;149(50):2795-9.

2) Gilpin BJ, Loechel F, Mattei MG, Engvall E, Albrechtsen R, Wewer UM. A novel, secreted form of human ADAM 12 (meltrin alpha) provokes myogenesis in vivo. J Biol Chem 1998 Jan 2;273(1):157-66.

3) Laigaard J, Sorensen T, Frohlich C, Pedersen BN, Christiansen M, Schiott K, et al. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. Prenat Diagn 2003 Dec 30;23(13):1086-91.

4) Wortelboer EJ, Linskens IH, Koster MP, Stoutenbeek P, Cuckle H, Blankenstein MA, et al. ADAM12s as a first-trimester screening marker of trisomy. Prenat Diagn 2009 Sep;29(9):866-9.

5) Spencer K, Cowans NJ, Uldbjerg N, Torring N. First-trimester ADAM12s as early markers of trisomy 21: a promise still unfulfilled? Prenat Diagn 2008 Apr;28(4):338-42.

6) Laigaard J, Sorensen T, Placing S, Holck P, Frohlich C, Wojdemann KR, et al. Reduction of the disintegrin and metalloprotease ADAM12 in preeclampsia. Obstet Gynecol 2005 Jul;106(1):144-9.

7) Spencer K, Cowans NJ, Stamatopoulou A. ADAM12s in maternal serum as a potential marker of pre-eclampsia. Prenat Diagn 2008 Mar;28(3):212-6.

8) Cowans NJ, Spencer K. First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system. Prenat Diagn 2007 Mar;27(3):264-71.

9) Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem 1983 Nov;21(11):709-20.