

MATERNAL SERUM MARKERS FOR DOWN'S SYNDROME PREGNANCIES

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ABSTRACT

Down's syndrome is the most common chromosomal abnormality whose incidence increases with advancing maternal age. However, approximately 70% of all Down's syndrome fetuses occur in mothers aged less than 35. Maternal serum markers have been used in an attempt to identify Down's syndrome pregnancies in these low risk mothers. Numerous second trimester maternal serum markers have been documented in the literature and these are reviewed. The Triple test which uses second trimester maternal serum levels of α -feto protein, human chorionic gonadotrophin and unconjugated oestriol is the most popular combination in use today. Although it is associated with a 58% detection rate for Down's syndrome pregnancies at a false positive rate of 5%, the Triple test has its problems and these are discussed. The cost-effectiveness of Down's syndrome screening using the Triple test and its role in mothers aged 35 years or more are also explored. Several workers have reported on first trimester serum markers of foetal Down's syndrome but more data is needed before a first trimester serum screening programme for Down's syndrome is possible.

Keywords: biochemical screening, Down's syndrome pregnancy.

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Down's syndrome (Trisomy 21) is still the most common human chromosomal abnormality, occurring in approximately one in 700 births⁽¹⁾. The risk of having a child with Down's syndrome increases with advancing maternal age⁽²⁾. From cost-benefit analyses, prenatal screening for Down's syndrome using maternal age seems to be cost beneficial at a risk cut-off point of approximately one in 200 (ie for women over 35)⁽³⁻⁵⁾.

However, only 30% of Down's syndrome pregnancies occur in women over 35 and screening based on maternal age alone has only resulted in a 15% reduction in the prevalence of births of Down's syndrome babies⁽⁶⁾. Although 70% of Down's syndrome babies are born to mothers less than 35 years old, until recently there has been no form of routine screening available. It has been recognised that the Down's syndrome pregnancy is associated with abnormal levels of maternal serum markers in the second trimester. The most extensively investigated serum markers include α fetoprotein (AFP), human chorionic gonadotrophin (HCG) and unconjugated oestriol (E_3). Other mid trimester markers that have been studied include free β -HCG, α -HCG, Schwangerschafts protein (SP1), CA-125 and inhibin.

α fetoprotein

AFP is a glycoprotein that is produced by the foetal yolk sac, liver and gastrointestinal tract^(7,8). In the maternal serum, levels of AFP rise progressively to reach a peak at 32 weeks before decreasing towards term⁽⁹⁾. It was Merkatz et al⁽¹⁰⁾ who first reported that maternal serum concentrations of AFP were

approximately 25% lower in the presence of foetal Down's syndrome compared to unaffected singleton pregnancies. Maternal serum AFP levels have been reported in 24 studies and the geometric mean for the affected pregnancies found to be 0.74⁽¹¹⁾. Although the association between low maternal serum AFP levels and Down's syndrome has not been fully elucidated, it is believed to be due to reduced foetal production^(12,13). This decrease in AFP concentrations has been shown to be independent of maternal age. Thus AFP measurements have been combined with a patient's age-related risk to arrive at an individualised risk for foetal Down's syndrome⁽¹⁴⁾. Studies using maternal serum AFP levels in combination with maternal age to screen for Down's syndrome, suggest that only 20-25% of Down's syndrome cases in younger women are identified⁽¹⁵⁻¹⁸⁾. However, a screening policy using the above combination has been assessed and still shown to be cost beneficial⁽¹⁹⁾. Furthermore, serum AFP screening has also been shown to be effective in detecting pregnancies at risk for open neural tube defects⁽²⁰⁾. Thus it may be the analyte of first choice in any second trimester prenatal screening programme.

Human chorionic gonadotrophin (HCG)

Human chorionic gonadotrophin is a glycoprotein made up of two subunits: an α subunit and a β -subunit⁽²¹⁾. It is produced by the placental syncytiotrophoblast and also possibly by the cytotrophoblast⁽²²⁾. Levels of HCG appear in maternal blood soon after implantation and increase rapidly until 8 weeks of gestation. Blood levels are little changed from 8-12 weeks, decline to 18 weeks and remain quite constant until term⁽²³⁾. Bogart et al⁽²⁴⁾ first described a raised maternal serum HCG associated with foetal Down's syndrome. Since then, this observation has been confirmed in many studies, and based on the results of 18 studies involving 559 affected pregnancies, the geometric mean level in the affected pregnancies was 2.05 multiples of the median for unaffected pregnancies of the same gestational age⁽¹¹⁾. Although the precise explanation for this finding is unknown, it may be due to fetuses with Down's syndrome being immature. Since maternal serum levels of HCG decline between 12-20 weeks of pregnancy, an immature foetus with Down's syndrome will be associated with a higher concentration of HCG compared to an unaffected pregnancy. At present, the single most discriminatory

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marker for Down's syndrome is the maternal serum HCG concentrations, which can detect 37% of women with affected foetuses at a risk cut-off that yields a 5% false positive rate⁽¹²⁾.

Unconjugated oestriol (UE₃)

Oestriol is produced by the syncytiotrophoblast from foetal precursors. It is secreted into the maternal circulation where levels rise progressively throughout gestation; paralleling the growth of the foetus and placenta. In maternal serum, it is measured as an unconjugated steroid and unlike total serum oestriol, the former is almost entirely derived from the foetus and placenta⁽²⁵⁾. Canick et al⁽²⁶⁾ first described low second trimester maternal serum UE₃ levels in pregnancies with Down's syndrome. From a summary of 11 studies based on 363 affected pregnancies, the geometric mean level in the Down's syndrome pregnancies was 0.73 multiples of the median for unaffected pregnancies of the same gestational age⁽¹¹⁾. The explanation behind the lower serum UE₃ levels in affected pregnancies is probably due to the immature foetuses with Down's syndrome. Low maternal serum UE₃ levels can be used to detect foetal Down's syndrome and at cut-off levels selected to pick up 35% of affected pregnancies, the marker was a better screening test than either maternal age or maternal serum AFP⁽²⁷⁾.

Free beta HCG

The free beta-subunit of HCG is found in maternal serum throughout pregnancy. However, reports on the amount of free beta-HCG in second-trimester maternal serum vary widely and range from 0.5% to 4.0% of the HCG concentration⁽²⁸⁻³¹⁾. Maternal serum levels of free beta HCG are raised in foetal Down's syndrome and the median values for affected pregnancies range from 2.0 to 2.41 MOM⁽³²⁻³⁵⁾. However, the role of free beta-HCG in antenatal screening for Down's syndrome is still controversial and unclear.

It has been suggested that the measurement of the free beta HCG instead of the total HCG molecule improves the performance of screening for Down's syndrome⁽³²⁻³⁵⁾. Furthermore, earlier reports of biochemical screening using free beta HCG have revealed detection rates of 68 to 92% of affected pregnancies with false positive rates of 3-5%⁽³⁶⁾.

However, in the paper by Macri et al⁽³²⁾, the mean and standard deviation of free beta-HCG (expressed in multiples of the median) for the Down's syndrome pregnancies were similar to the values for total HCG. The overlap in affected and unaffected pregnancies were also similar for free beta HCG and total HCG. Thus the performance of the two tests are unlikely to be different⁽¹¹⁾. Knight et al⁽³⁷⁾ compared the use of free beta HCG and total HCG in screening for foetal Down's syndrome and concluded that the two forms were just as effective.

Estimates of the actual concentrations of free beta HCG in the second trimester show considerable variability and this may be due to the use of immunoassays with different cross-reactivities with HCG. This may pose difficulties in obtaining accurate measurements of free beta subunits in the presence of high concentrations of HCG. There is also evidence that the proportion of free beta subunit increases with the duration of storage of serum samples⁽³⁷⁾. There is generally less practical experience with free beta HCG and also a lack of data on its use in pregnancies with twins, insulin dependent diabetes mellitus and those in which gestational age is estimated by ultrasound. Thus, until more data is available, routine substitution of free beta HCG measurement for the better established HCG assay cannot be justified.

Free α subunit HCG

Bogart et al^(24,38) reported a median value of 2.05 MOM in affected

pregnancies and suggested that the use of α -subunit improves detection rates by 15%. However, more work needs to be done before its role in biochemical screening can be defined.

Other serum markers

Maternal serum pregnancy specific beta-1 glycoprotein (SP-1) is a nonhormonal protein produced by the syncytiotrophoblast of the placenta. Bartels et al⁽³⁹⁾ were the first to report raised maternal serum SP-1 levels associated with foetal Down's syndrome. Based on a total of 5 studies with 213 affected pregnancies, the geometric mean level in the affected pregnancies was 1.54 multiples of the median⁽¹¹⁾. However, workers have reported that the addition of SP1 levels would increase the detection rate by less than 3% and thus would not justify its use in antenatal screening for Down's syndrome⁽⁴⁰⁾.

Cancer antigen 125 (CA125) is an antigenic determinant on a high molecular weight glycoprotein. The physiological role of the glycoprotein is as yet unknown. There have been suggestions that maternal CA125 levels may be associated with foetal aneuploidy⁽⁴¹⁾. However, Blerk et al⁽⁴²⁾, in a retrospective analysis of maternal serum samples from ten Down's syndrome pregnancies and 78 controls, could not find a significant association between Down's syndrome and second trimester maternal serum CA125 levels.

Van Lith et al⁽⁴³⁾ measured immunoreactive inhibin in maternal serum of ten Down's syndrome pregnancies and in 80 normal pregnancies in the second trimester. They found that inhibin levels in the Down's syndrome pregnancies were significantly higher than in the unaffected pregnancies. However more work is needed before immunoreactive inhibin is used as a marker for foetal Down's syndrome.

Other serum markers that have been reported on include human placental lactogen, progesterone, and thyroid antibody levels⁽⁴⁴⁻⁴⁶⁾. However, their roles are yet to be defined.

The Triple Test

At present, biochemical screening using second trimester maternal serum measurements of AFP, HCG and UE₃ together with maternal age (triple test) is the method of choice. This method of risk estimation devised by Wald et al⁽¹²⁾ involves the age-specific risk, as an odds ratio, being multiplied by the likelihood ratio derived from the appropriate trivariate Gaussian frequency distributions of the serum markers. Although the triple test may be performed between 15 and 22 weeks of pregnancy, it is best done at 15-16 weeks as this allows the simultaneous assessment of AFP as an additional screen for neural tube defects.

The value of UE₃ as an additional marker to AFP and HCG has generated much discussion. Macri et al⁽³²⁾ concluded that the measurement of UE₃ was not useful because the study failed to find an association between low UE₃ levels and Down's syndrome. However, other studies⁽¹¹⁾ have confirmed the presence of lower maternal serum UE₃ levels in Down's syndrome pregnancies. Some workers have shown a small improvement in the predicted detection rates for Down's syndrome with a corresponding decrease in the false positive rate^(12,47); whereas others have shown an increase in detection only at the expense of an increase in the false positive rate⁽⁴⁸⁾. Other problems include the fact that oestriol measurements are technically more difficult to perform compared to AFP and HCG. Furthermore, of the 3 analytes, UE₃ is also the most affected by inaccuracies in the gestational age.

Wald et al⁽¹²⁾ reported that the Triple test, using a risk cut-off of one in 250 at term, could detect 61% of Down's syndrome pregnancies with a 5% false positive rate. This estimated detection rate has since been revised from 61% to 58%⁽⁴⁹⁾. Other workers have also confirmed the efficacy of screening for Down's

syndrome using the Triple test⁽⁴⁸⁾.

However, the use of the Triple test in prenatal screening for Down's syndrome is not without its problems. Screening may be associated with adverse psychological consequences, but these may be avoided if patients are counselled before screening and supplemented with written material⁽⁵⁰⁾. Informed consent is also required. The patient should be told what the test is for, its false positive and false negative rates. The consequences of a positive result must also be explained especially since the loss of a normal foetus may occur as a complication of the diagnostic procedure⁽⁵¹⁾. Although obtaining informed consent is time consuming and increases costs, it is medico-legally important as "failure to obtain informed consent for a screening programme is not only ethically unacceptable but also exposes the health authority to the risk of litigation⁽⁵¹⁾".

Maternal anxiety can also be generated by triple test screening, not to mention the stress associated with amniocentesis and late terminations. We must also consider the negative effects of false positive results on maternal attitudes towards the baby, both before and after delivery as well as the unknown effects of maternal stress on foetal development⁽⁵²⁾.

The performance of the triple screening test depends on an accurate estimation of gestational age. Estimation of gestation by the last normal menstrual period in women who were sure of their dates have been shown to result in an error of more than two weeks in 17% of women⁽⁵³⁾. Furthermore, the error seems to be greater in the younger age groups⁽⁵⁴⁾ which form the bulk of women in the screening programme. However, the routine use of ultrasound for gestational age estimation has been shown to reduce the random error associated with the calculation of the serum marker values in multiples of the median for a given gestational age. Detection rates, when gestational age is based on foetal biparietal diameter measurements in the second trimester, are 67% compared to 58% when based on the last menstrual period⁽⁴⁹⁾.

The biparietal diameter is the ultrasound parameter of choice because Down's syndrome foetuses have mean biparietal diameters that are identical to those of unaffected foetuses⁽⁴⁹⁾. Its use is also advantageous in AFP screening for neural tube defects as it increases the detection rates for open spina bifida⁽⁵⁵⁾. It has also been reported⁽⁵⁶⁾ that selective use of ultrasound scans among women with positive screening results can lead to a lower detection rate. Thus if ultrasound estimation of gestational age is used, it should be done routinely for all women and not selectively for those with positive results.

Many obstetric units perform routine foetal anomaly scans at 18-19 weeks and since biochemical screening for Down's syndrome is performed earlier at 16 weeks, it has been suggested that an earlier scan may be required to date all pregnancies for the purposes of screening⁽⁴⁹⁾. This will surely increase the logistical and financial burdens of screening. Serum markers of the triple test are also affected by maternal weight, twin pregnancies and women with insulin-dependent diabetes mellitus. Increasing maternal weight is associated with a lowering of maternal serum AFP, UE₃ and HCG levels. Wald et al⁽⁴⁹⁾ have reported that routine maternal weight adjustments for the serum marker levels can increase the detection rate by approximately 0.5%, for a given false positive rate or reduce the false positive rate by 0.1%, for a given detection rate.

In twin pregnancies, the median maternal serum AFP is 2.1 times as high, the median UE₃ levels 1.7 times as high and the median HCG levels 1.8 times as high as in singleton pregnancies. In women with insulin-dependent diabetes mellitus, the maternal serum AFP, UE₃ and HCG levels are 0.7, 0.92 and 0.95 multiples of the median respectively^(57,58). Unfortunately, the distributions of α FP, HCG and UE₃ values in diabetic and twin pregnancies

affected by Down's syndrome are not known. Thus the risk of Down's syndrome in such pregnancies cannot be determined directly. However, by dividing AFP, HCG and UE₃ multiples of the median values for non diabetic and singleton pregnancies by the corresponding median values for diabetic and twin pregnancies, the risk of Down's syndrome may be estimated.

When Wald et al⁽¹²⁾ reported on the effectiveness of the triple test, their calculations were based on measurements made on stored serum samples from Down's affected pregnancies. This however, is very different from identifying foetuses with Down's syndrome in a random sample of pregnant patients. Wald et al⁽⁵⁶⁾ reported on the results of their demonstration project conducted in four health districts in London. Involving more than twelve thousand women, screening uptake was 74% and the uptake of amniocentesis in screen positive women was 75%. They reported a detection rate of 48%, a false positive rate of 4.1% and concluded that maternal serum screening for Down's syndrome could be carried out effectively on a community basis, as a routine part of antenatal care. However, other workers⁽⁵⁹⁾ have pointed out that the detection rate of 48% in that study was considerably below the 58% previously suggested⁽⁴⁹⁾. Furthermore, the detection rate of only 39% in women aged under 37 in whom most affected pregnancies occur has been described as less than impressive⁽⁶⁰⁾.

However, in a similar demonstration project performed in the United States, Haddow et al⁽⁶¹⁾ prospectively screened more than 25,000 women in the second trimester of pregnancy and reported a 58% detection rate for foetal Down's syndrome and a false positive rate of 3.8%. Philips et al⁽⁶²⁾ also prospectively evaluated the triple test in 9,530 women and reported a sensitivity of 57% with a false positive rate of 3.2%.

The cost-effectiveness of Down's syndrome screening using the triple test has been examined by several workers. Shackley et al⁽⁶³⁾ evaluated economically, a triple test screening programme in the Oxford Region and concluded that the most efficient detection rate was around 58% for which the cost per Down's birth avoided was £49,800. Sheldon et al⁽⁶⁴⁾ appraised the cost effectiveness of the triple test screening programme in Leicestershire. They reported that the most efficient detection rate was around 60-65%, for which the cost per case detected was approximately £29,000. However, cost effective calculations are dependent on the rates of uptake of both amniocentesis and termination. Furthermore, the ethical and emotional issues associated with a screening programme aimed at preventing the birth of "imperfect individuals" cannot be addressed by cost effective studies alone.

The role of serum screening for Down's syndrome in pregnant women of advanced maternal age (ie aged 35 years or more) is somewhat contentious. It has always been established practice for all pregnant women aged 35 years and above, to be offered amniocentesis and many screening programmes are reluctant to interfere with this precedent. However, Wald and Cuckle⁽¹²⁾ contend that the triple test offers a cost effective form of screening, especially if it is carried out on all pregnant women, regardless of their age. For a 5% amniocentesis rate, the Down's syndrome detection rate would be 58% if serum screening were offered to all women but only 49% if amniocenteses were offered to women aged 37 or more, and serum screening restricted to younger women. Furthermore, the triple test compared to maternal age alone, seems to offer a more accurate estimate of a patient's risk of a Down's syndrome pregnancy. Wald et al⁽⁵⁶⁾ reported that the older mothers who underwent serum screening, were willing to avoid amniocentesis if their risks were low. However, in retrospective studies, the reported sensitivities of serum screening in women aged 35 years and above have varied widely. Mancini et al⁽⁶⁵⁾ in a study involving 731 patients aged

35 years or more who had triple test screening, reported that all 9 affected pregnancies (cut-off risk levels of 1:250) were identified. In addition, serum screening would also reduce the number of amniocentesis to a third without significantly affecting detection. Wald et al⁽¹²⁾ reported that using a cut-off level of 1:250, the detection rate in women over 35 years was 86%. Heyl et al⁽⁶⁶⁾ identified 75% of Down's syndrome cases when they analysed stored sera of women aged 35 years or more.

Prospective studies seem to yield more consistent results. MacDonald et al⁽⁴⁸⁾ found that with triple test screening in women aged 35 years or more, 75% of foetal Down's syndrome could be detected with an amniocentesis necessary in only one in five women. Wald et al⁽⁵⁶⁾ also reported a 75% detection rate with prospective triple test screening in patients aged 37 years or more.

However, triple test screening may not detect other chromosomal abnormalities. Heyl et al⁽⁶⁶⁾ reported that only 3 of 18 cases of aneuploidies other than trisomy 21 would have been picked up using triple test screening. Although risk assessment by serum screening in older women appear promising, it will miss some pregnancies with Down's syndrome or other chromosomal abnormalities, that would have been picked up had amniocentesis been performed universally. Thus, older women should be counselled that triple test is not a diagnostic procedure and that it can miss 20-30% of foetal Down's syndrome as well as a significant proportion of other chromosomal abnormalities.

More recently, the "triple plus" test⁽⁶⁷⁾ has been described which incorporates urea-resistant neutrophil alkaline phosphatase as the fourth second trimester serum marker. Grozdea et al^(68,69) were the first to report raised activities of urea resistant neutrophil alkaline phosphatase in pregnant women with foetal Down's syndrome and in women who in the past had had a Down's syndrome pregnancy. The median enzyme activity in Down's syndrome pregnancies have been reported to be 1.65 times the median in controls of the same gestational age⁽⁷⁰⁾. However, the mechanism behind this raised activity in affected pregnancies is still unknown. Cuckle et al⁽⁷⁰⁾ reported that when a cut off value of 1.40 multiples of the normal median was used, 79% of the affected pregnancies were detected with a false positive rate of 5%. These results suggest that the urea-resistant neutrophil alkaline phosphatase test is presently the most discriminatory blood test for Down's syndrome in the second trimester.

However, problems do exist with its use. The test is labour intensive and subjective as it relies on the assessment of the degree of staining of cytoplasmic granules in the neutrophil. Initial data⁽⁷⁰⁾ has so far been retrospective in nature and has included older women. More information is also needed in order to assess the test's performance at 15-16 weeks as well as its correlation with the other 3 existing serum markers. Thus more work needs to be done before the test is made available for routine clinical use.

It should also be noted that some authors have proposed the use of different combination of markers in various screening protocols. These have included the use of human chorionic gonadotrophin and AFP⁽⁷¹⁾; human chorionic gonadotrophin and pregnancy specific β 1 glycoprotein⁽⁷²⁾; the ratio of HCG to AFP⁽⁷³⁾ and many others. However, more data is needed before their role in serum screening is defined.

Serum screening in the first trimester

Although there is still at present no reliable test for serum screening in the first trimester, there have been reports of maternal serum markers being associated with a Down's syndrome pregnancy in the first trimester.

Low maternal serum AFP values in the first trimester have been shown to be associated with foetal Down's syndrome⁽⁷⁴⁾.

The weighted AFP median from 12 studies based on 136 patients with foetal Down's syndrome was 0.77⁽⁷⁵⁾. Down's syndrome pregnancy is also associated with low maternal serum oestriol levels in the first trimester. The median calculated from 3 studies and 48 affected pregnancies was 0.54⁽⁷⁵⁾.

However, workers have reported that maternal serum human chorionic gonadotrophin levels in the first trimester were not useful in detecting pregnancies at risk for foetal aneuploidy^(38,76,77). Bogart et al⁽³⁸⁾ also measured free alpha-human chorionic gonadotrophin levels in first trimester pregnancies with foetal chromosomal abnormalities and found that these were not significantly different from normal controls.

There have been reports that maternal serum cancer antigen 125 (MS-CA125) may be associated with foetal Down's syndrome. In normal pregnancies, the MS-CA125 levels increase between 5 and 11 weeks of gestation before dropping in the second trimester⁽⁷⁸⁾. Hogdall et al⁽⁴¹⁾ found that MS-CA125 levels in the first trimester were elevated in Down's syndrome pregnancies compared with controls. They concluded that MS-CA125 was a better serum marker than AFP for a Down's syndrome foetus in the first trimester. Van Lith et al⁽⁷⁹⁾, on the other hand, reported that levels of MS-CA125 in the first trimester were significantly lower in women with foetal Down's syndrome compared to unaffected pregnancies. More work is thus needed to clarify the situation.

Pregnancy-associated plasma protein A (PAPP-A) is produced by the placental trophoblast and reduced levels in the first trimester have been associated with Down's syndrome fetuses^(80,81). The mechanism behind the reduced levels in affected pregnancies is uncertain but may be due to a general decrease in trophoblastic function. Brambati et al⁽⁸²⁾ in a retrospective study of PAPP-A levels in the first trimester, found that the median value of PAPP-A in the abnormal pregnancies was 0.27 multiples of the normal median which was significantly lower when compared with the normal pregnancies. They suggest that screening with PAPP-A levels may identify 60% of cases of Down's syndrome in the first trimester with a false positive rate of 5%. More prospective data is needed to evaluate its role in first trimester serum screening.

Pregnancy-specific beta-glycoprotein levels have been investigated and Brock et al⁽⁸³⁾ have reported that both pregnancy-specific beta-glycoprotein and maternal serum human chorionic gonadotrophin may be useful as a first trimester screen for Down's syndrome.

Nebiolo et al⁽⁸⁴⁾ using first trimester maternal serum AFP and human chorionic gonadotrophin subunit ratios found that a third of fetuses with Down's syndrome and 83% of cases with trisomy 18 could be detected at a karyotyping rate of 8.6%.

Kratzer et al⁽⁸⁵⁾ describes an aneuploidy index using first trimester serum levels of progesterone, alphasubunit and total human chorionic gonadotrophins. They report a detection rate of 35% of trisomy 21 pregnancies with a false positive rate of 4.5%.

Although many workers have reported on various serum markers for Down's syndrome pregnancies on the first trimester, it still remains that the numbers of sera from affected and unaffected pregnancies are still too low to permit an introduction of a first trimester serum screening programme for Down's syndrome⁽⁷⁶⁾.

Conclusion

Maternal serum screening was born out of the need to identify the 70% of Down's syndrome fetuses that occur in the younger age group mothers. Many second trimester serum markers have been studied but the triple test, which employs the use of serum AFP, HCG, UE₃ and maternal age, is still the most popular

combination. Although it promises a 58% detection rate of Down's syndrome pregnancies at a false positive rate of 5%, the triple test is not without its problems. More prospective studies are also needed to evaluate its performance in the screening of antenatal populations. The use of urea-resistant neutrophil alkaline phosphatase as a fourth analyte appears promising but more work needs to be done. Although the initial data on first trimester serum markers of foetal Down's syndrome is encouraging, more information is required before a first trimester screening programme for Down's syndrome becomes a reality.

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