Technical Brief

Prenatal Quad Screen

Background Information
Trisomy 21 [Down syndrome] occurs in 1 in 800 live births. Currently, Down syndrome screening involves the measurement of multiple biochemical markers during the early second trimester, between 15-20 weeks of pregnancy. Along with the age of the woman, levels of these biomarkers in the maternal blood have been used to identify the high-risk pregnancies. Initially a panel of three markers was used that included unconjugated estriol (uE3) and human choriogonadotropin (HCG), and alpha Feto-protein (AFP), known as Triple Marker Screen.

Later some studies found a fourth marker, dimeric inhibin-A [DIA] to be useful in assessing risk and further enhancing sensitivity of prenatal screening. The inhibins are a family of glycoprotein hormones that are produced by the placenta and ovaries in two forms: Inhibin A consisting of an alpha and a beta-A chain; and Inhibin B which has two beta-B chains. Based on published data, second trimester levels of inhibin A in maternal serum are about two times higher in Down syndrome. The addition of inhibin A to the triple marker panel led to the generation of the “Quad Marker” panel, which is the updated version of laboratory tests designed to determine risk for open neural tube defects, Fetal Down’s Syndrome (FDS) and trisomy 18, during the second trimester of pregnancy. The combination of DIA, AFP, uE3, hCG, and maternal age will detect about 75% of Down syndrome affected pregnancies while maintaining a false positive rate of about ~4%, which is a slightly less false positive rate than heretofore seen with the Triple marker screen. All other aspects of prenatal maternal serum screening are the same for both the Triple Marker screen and the Quad Marker screen.

Clinical Information
Quad marker screening has become a standard tool to identify pregnancies that may have an increased risk for certain birth defects, including neural tube defects (NTDs), Down syndrome, and trisomy 18. For risk calculations, patient results are compared to population-based median values and reported as multiple of medians (MoM). The MoM value for each marker contributes to the calculated risk for affected pregnancy. Risk assessment is performed using a statistical algorithm known as multivariate Gaussian distribution analysis. Adjustments to MoM values are made for maternal weight, race, number of fetuses and maternal insulin dependent diabetes. Therefore, for accurate risk assessment it is necessary to have all these pieces of information provided correctly on the requisition form. Ultrasound dating will be used for calculation of gestational age whenever it differs from last menstrual period (LMP) dating by 11 days or more. Recalculation of results is performed upon request with updated information. The optimal window for testing is between 16-18 weeks of gestation. The laboratory has defined MoM values for 15-22 weeks gestational age. The Quad Marker Screen should be redrawn if gestational age was less than 15 weeks when the initial specimen was drawn.

Methodology
The screen is performed by measuring four analytes (AFP, uE3, HCG & Inhibin A) in maternal serum that are produced by the fetus and the placenta. They are measured by chemiluminescence immunoassays on automated platforms. Cut-off levels for these as defined in the laboratory are listed below.

- **Neural Tube Defects**
A calculated MoM value of < 2.0 for AFP is defined as screen negative.

- **Down Syndrome**
Calculated risks = or < 1/270 are reported as screen negative, risks > or = 1/270 are reported as screen positive.

- **Trisomy 18**
Calculated risks < 1/100 are reported as screen negative, risks > or =1/100 are reported as screen positive.

Interpretation
The analyte values along with maternal demographic information such as age, weight, gestational age, diabetic status, and race are used together in a mathematical model to derive a risk estimate.

A specific cutoff level for each condition has been established, which classifies each screen as either screen-positive or screen-negative. A positive screen does not provide a diagnosis, but indicates the increased risk and the need for further evaluation.
Limitations

Several variables like race, weight, multiple fetus pregnancy and insulin-dependent diabetes (IDD) are known to affect marker concentrations. Therefore, estimated risk calculations and screen results are dependent on accurate information for these factors. Inaccurate information of gestational age, for example, can result in false-positive or false-negative screen results. Due to its increased accuracy, determination of gestational age by ultrasound is recommended, rather than by last menstrual period.

Prenatal screen is not a diagnostic test but rather a tool for risk assessment. A negative test does not necessarily rule out the absence of fetal defects and a positive test is not diagnostic but suggests that further testing should be performed.

Table 1: Markers trend in relation to increase risk for ONTD, DS and Trisomy 18

<table>
<thead>
<tr>
<th></th>
<th>AFP</th>
<th>uE3</th>
<th>Total HCG</th>
<th>Inhibin A</th>
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<td>↑</td>
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<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Down Syndrome</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Trisomy 18</td>
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References