The recent introduction of the option for screening for the 22q11.2 deletion has prompted a review of the Harmony Prenatal Test report format. Some minor report changes have been made to improve the clarity of information provided to referring doctors, and to avoid potential confusion between the FORTE algorithm probability value (detailed below) and the post-test probability of a fetal chromosomal abnormality.

FORTE produces a probability estimate for presence of each screened chromosomal aneuploidy, based on all of the factors outlined above.1-4 The majority of low-risk FORTE scores are <0.01%, and the majority of high-risk scores are >99%. To ensure the highest test detection rate and lowest false-positive rate, a cut-off FORTE probability value of 1% is used to differentiate high-risk Harmony results from low-risk results.

This cut-off is applied to all Harmony tests. It was used in large clinical studies to determine the test performance characteristics — i.e. detection rate and false-positive rate. These test performance characteristics are provided in tabular form on each Harmony report, for each screened abnormality.

FORTE algorithm probability value

FORTE (Fetal-Fraction Optimised Risk of Trisomy Evaluation) is the algorithm at the heart of the Harmony Prenatal Test.1,2 Targeted fragments are amplified from chromosomal regions of interest using DANSR (Digital Analysis of Selected Regions) assays.3 The FORTE algorithm considers the relative intensity of DANSR assay signals, along with variance ('noise') in the data, and other measurements of data quality.

FORTE also incorporates maternal risk factors, the number of fetuses and, most importantly, the measured fetal fraction. In the new Harmony report, demographic information considered by the FORTE algorithm is grouped together in the top right-hand corner of the report. This is coloured in blue rather than black, to highlight that it is important to check the accuracy of this information (estimated gestation, IVF status and the number of fetuses).
Probability of a chromosomal abnormality in the fetus versus FORTE probability value

The FORTE probability value reflects the odds that the tested cell-free DNA (cfDNA) sample is either trisomic or euploid. However, it does not directly provide the probability of whether the fetus is trisomic or not.

This is because cfDNA is derived from the placenta, and there are a number of biological factors which can cause discordance between the genetic status of cfDNA and the fetus. Examples include confined placental mosaicism, fetal mosaicism, maternal chromosome changes, and the presence of an unrecognised non-viable twin.

As with any screening test, the post-test probability that the fetus is affected when a non-invasive prenatal test returns a high-risk result will depend on two factors:

1. Test performance characteristics (detection rate/false-positive rate) – these are provided on each Harmony Prenatal Test report, and
2. The pre-test probability of the tested abnormality.

The higher the pre-test probability, the higher the likelihood that a high-risk cfDNA result will reflect fetal genetic status. Conversely, in a patient with low pre-test probability, the likelihood that a high-risk cfDNA result reflects fetal genetic status is reduced.

Post-test probability for a high-risk result is sometimes known as the Positive Predictive Value (PPV) of the test — although PPV is a population-based statistic rather than an individualised post-test risk score.

In the same way, the likelihood that a fetus is unaffected when a non-invasive prenatal test returns a low-risk result will depend on both test performance characteristics, and on the pre-test probability of an abnormality. The risk of a false-negative result is low in all risk categories, but it is increased if there is a very high pre-test probability of a chromosomal abnormality — e.g. risk >1/10 on the combined first trimester screen, or presence of a major fetal structural abnormality on ultrasonography.

In addition, if clinical factors indicate a very high risk of a chromosomal abnormality, the possibility should be considered of an atypical abnormality, which is not screened for by Harmony but may be detected by invasive testing (chorionic villus sampling or amniocentesis).

New report format — Clear presentation of high- and low-risk results

The FORTE probability score was previously provided on Harmony reports as additional information. However, this could possibly be misinterpreted as test PPV for high-risk results, or test NPV for low-risk results.

For this reason, the new Harmony report does not provide the FORTE probability score, and instead states whether the result is high-risk (i.e. FORTE probability value >1%) or low-risk (FORTE probability value <1%) for each tested abnormality.

The post-test probability in the light of a high-risk (or low-risk) result will vary with the patient-specific pre-test probability, rather than the exact value of the FORTE probability score. If the FORTE probability value is still required for a particular patient, please contact the local Sonic Genetics laboratory.

In the new report format, an indicative PPV value from an Australian obstetric cohort tested by Harmony is provided for high-risk results (where data is available for that abnormality). For low-risk results, an example of estimated NPV, assuming a prior probability of 1/50 and 1/500, will be provided (NPV will be higher for patients at lower prior risk, and vice versa).

Further information on post-test probability is available on the Sonic Genetics website:

References