Non-invasive prenatal testing (NIPT) for 22q11.2 deletion syndrome using a targeted microarray-based cell-free DNA (cfDNA) test

Maximilian Schmid[1], Eric Wang[1], Patrick Bogard[1], Jacob Zahn[1], Coleen Hacker[1], Susie Wang[1], Jigna Doshi[1], Karen White[1], Jennifer Kaplan[1], Annette Batey[1], Andrew Sparks[1], Jaques Jani[2], Renee Stokowski[1]

[1]Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc., [2]Department of Obstetrics and Gynecology, University Hospital Brugmann, Université Libre de Bruxelles, Brussels, Belgium

Objective: Cell-free DNA (cfDNA) testing provides high sensitivity for common trisomies and a lower false positive rate than traditional prenatal screening methods. Recently, the scope of testing has expanded to include subchromosomal deletions. Here we aim to determine the performance of a targeted microarray based cfDNA test (Harmony Prenatal Test®) to screen for pregnancies at increased risk for a 22q11.2 deletion.

Methods:

Test performance was determined in two steps. First, analytical validation for detection of 22q11.2 deletion was performed in 1736 plasma samples, 122 of which had a 22q11.2 deletion ranging in size from 1.96 to 3.25 Mb. These included simulated pregnancy samples and maternal plasma from pregnancies with a fetal 22q11.2 deletion. The simulated samples were created with plasma from individuals with 22q11.2 deletions titrated with plasma from unaffected, non-pregnant women to simulate fetal fractions ranging from 4% to 33%. The remaining 1614 samples were from women with presumed unaffected pregnancies in which the maternal and fetal 22q11.2 deletion status was unknown.

In the second stage, clinical verification of performance was performed in 217 samples from singleton pregnancies with known 22q11.2 deletion status. Seven samples were from pregnancies with a 22q11.2 deletion confirmed by FISH or microarray analysis, and 210 were from unaffected pregnancies confirmed by normal chromosomal microarray analysis.

Cell-free DNA analysis using Digital ANalysis of Selected Regions (DANSR™) was performed as previously described with additional assays for assessment of fetal 22q11.2 deletions within a 3.0 Mb region. DANSR products were quantified on custom microarrays and the probability of a deletion being present was determined using the FORTE™ algorithm.

Results:

Of 122 samples in the analytical validation set with a 22q11.2 deletion, 92 were found to have a high probability of a deletion (sensitivity: 75.4%; 95% CI: 67.1-82.2%). No evidence of a deletion was observed in 1606 of 1614 samples from presumed unaffected pregnancies (specificity: 99.5%; 95% CI: 99.0-99.7%).

In the clinical verification cohort, 5 of 7 samples from pregnancies affected with 22q11.2 deletion were determined to have a high probability of deletion. There were no false positive results in the 210 unaffected samples in this cohort. The data from the clinical verification set is consistent with the performance demonstrated in the analytical validation.

Figure 1. 22q11.2 chromosomal region and location of DANSR assays

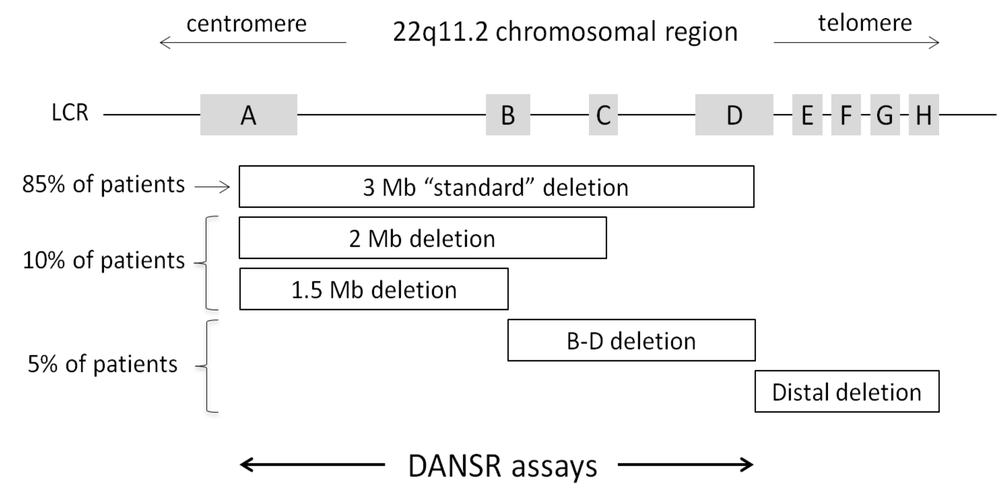

Table 1. Sensitivity and specificity of 22q11.2 deletion analysis using targeted microarray cfDNA testing

	Analytical validation	Clinical verification	Combined
Total samples (N)	1736	217	1953
22q11.2 (n/N)	92/122	5/7	97/129
No evidence of a deletion (n/N)	1606/1614*	210/210	1816/1824
Sensitivity % (95% CI)	75.4 (67.1-82.2)	71.4 (35.9-91.8)	75.2 (67.1-81.8)
Specificity % (95% CI)	99.5 (99.0-99.7)	100 (98.2-100)	99.6 (99.1-99.8)

*Presumed normals - commercial samples without a known diagnosis of 22q11.2 deletion, presumed to be unaffected (may cause underestimation of specificity)

Conclusions:

Targeted cell-free DNA testing using microarray quantitation is able to identify pregnancies at increased risk for 22q11.2 deletions of 3.0 Mb and smaller while maintaining a low false positive rate.

Disclosure: MS, EW, PB, JZ, CH, SW, JD, KW, JK, AB, JS, AS, and RS are employees of Ariosa Diagnostics Inc., and Roche Sequencing Solutions Inc.

MA-00794 Rev 2.0 04/28/2017