SEQUENOM™

A Review of Trisomy 21 Testing: Laboratory Developed Validation and Regulatory Considerations

Background on Trisomy 21 Testing:

Down syndrome (DS), also called Down syndrome Syndrome and Trisomy 21, is a condition in which extra genetic material causes delays in the way a child develops, both mentally and physically. It affects about 1 in every 733 babies. The American College of Obstetricians and Gynecologists (ACOG) now recommends that all pregnant women be offered screening with the option for invasive diagnostic testing for Down syndrome, regardless of age.

(http://www.acog.org/from_home/publications/press_releases/nr01-02-07-1.cfm)

Of the 4.3 million births annually in the US, it is estimated that approximately 70% (3.0 million) accept prenatal risk assessment (screening). Screening programs typically designate between 5-10% (~8% average) of all patients screened as being high-risk and refer them for an invasive testing procedure, either amniocentesis or chorionic villus sampling (CVS), which carries with it a risk of miscarriage.

Several **Screening** tests are currently available. Serum marker analysis (i.e. blood tests), such as the Quad Screen, has typically been offered between the 15th and 20th week of pregnancy to screen for Down syndrome and, to a limited extent, other chromosomal disorders. This second trimester test has a detection or sensitivity rate of 80%, meaning that 20% of all affected pregnancies will not be detected for further evaluation. In addition, the false positive rate for the Quad screen ranges between 5 to 10%, depending on the accuracy with which gestational age was determined. These false positive tests result in hundreds of thousands of unnecessary referrals annually for confirmation by amniocentesis.

Another **Screening** approach that has gained favor in an effort to improve detection rates is 1st Trimester Combined Screening, which combines two serum markers, PAPP-A and hCG, with a specialized fetal ultrasound measurement called Nuchal Translucency (NT). This screening approach is performed between the 10th and 13th week of pregnancy but has only resulted in an increase of 5-7% in the sensitivity for Down syndrome compared to the current Quad Screen. 1st Trimester Combined Screening still has the same 5-10% false positive rate as Quad and similarly depends on accurate estimation of gestational age. While the collection and testing of blood is relatively simple, the performance of the NT procedure requires specialized training. As such, the 1st Trimester Combined Screen is not widely available to all patients in the United States; only 20% of obstetrics practices offer NT ultrasound. Patients who are screened using the 1st trimester combined test and told that they are high-risk may then choose to undergo either Chorionic villus sampling (CVS), which may only be performed during a very specific time window in the 1st trimester, requires greater operator expertise, and is not widely available, or amniocentesis in the second trimester. Since amniocentesis is a more commonly available procedure and therefore

better suited for screening tests that yield results in the 2nd trimester, many women and physicians do not bother with 1st trimester combined screening.

Irrespective of which screening test is performed, the indication by a screening test of a high risk of Down syndrome leads to an invasive, **diagnostic (confirmation)** test which is recommended to pregnant patients to determine whether the fetus actually has Down syndrome. These invasive procedures carry a risk of miscarriage on the order of 1 in 100 to 1 in 300.

What is the SEQureDx™ Technology?

Sequenom's development efforts with the SEQureDx Technology may provide a potentially practice changing approach to prenatal screening for Down syndrome.

Upon successful validation, Sequenom, Inc will offer its circulating cell-free fetal nucleic-acid (ccfNA) technology, called SEQureDx, as a Laboratory Developed Test (LDT). The SEQureDx test for trisomy 21 identifies fetal-specific transcripts, each containing multiple single nucleotide polymorphisms (SNPs) that allow for gene-dosage analysis using Sequenom's MassARRAY® technology. SEQureDx is a direct measurement of the number of copies of chromosome 21. It can yield an accurate answer for any woman whose fetus is heterozygous (i.e. different) for at least one of a dozen identified SNPs on Chromosome 21 and where enough RNA material is present in the collected blood sample. In a normal heterozygous sample, the measured ratio is 1:1 (one chromosome from the mother and one chromosome for the father), whereas, in a Down syndrome positive case the data is reported as a ratio of 2:1, signifying an extra copy of chromosome 21.

The test will have an unprecedented number of safety features. For example, it will identify homozygotes (i.e. SNPs are all the same) and patients with an insufficient RNA copy number. Sequenom currently estimates that about 5-7% of all fetuses will show up as homozygous for all SNPs and these women will be deemed "no-calls" and reflexed to an alternative test such as Quad of Integrated. Heterozygous patients with inadequate RNA copy number in their sample will be deemed "no-calls" and will have the option of either being reflexed to an alternative test or may resubmit a blood sample a short while later that will likely have enough material to yield an accurate answer. The key is that in no case will a no-call patient receive any kind of answer about the status of her pregnancy; it would be as if the test had never been performed, which is why a "no-call" does not factor into the accuracy of the test.

Sequenom, Inc has demonstrated through initial research studies on now over 400 pregnancies that their circulating cell free nucleic acid (ccfNA) technology SEQureDx has, to date, detected all Down syndrome cases with no false-positive results. The key attributes of the SEQureDx Technology are that it is a Direct test vs. a Surrogate test, can be performed in either the 1st or 2nd trimester of pregnancy, is highly sensitive, has a very low false-positive rate, does not suffer from error caused by inaccurate determination of gestational age, and yields a straight-forward "yes/no" answer instead of a risk-score (e.g. high-risk vs. low-risk).

When will the SEQureDx Trisomy21 Technology be available?

The SEQureDx T21 technology will be evaluated as a Laboratory Diagnostic Test (LDT) through Sequenom's recently acquired CLIA certified laboratory. Current plans are that sufficient analytical and clinical validation of the LDT method will enable the commercial launch of the technology in the first half of 2009. The CLIA launch will be based on results of a prospective validation study 3-5,000 samples, to be completed before June 2009. To date, results from more than 400 samples have demonstrated that the SEQureDx fetal RNA-based test appears to exhibit high clinical performance (i.e., 100% detection rate and 0% false positive rate), having detected 6 positive Trisomy 21 cases without any false positive results. SEQureDx Trisomy 21 test has reached a high degree of coverage, 93-95%, in the general population (i.e. 93-95% of the population is expected to be heterozygous for at least one SNP and therefore the test can yield an accurate answer as long as adequate RNA is present in the sample) with a false-positive rate that is very low (<1% at a 95% confidence limit). In heterozygous pregnancies, sensitivity approaches 100%. The overall coverage is expected to be increased to greater than 95% prior to the test launch in June 2009 while maintaining its accuracy. Based on only 400 samples, the test's false-positive rate is already in the same ultra-low range as invasive procedures such as CVS and Amniocentesis. Larger 3-5,000 sample studies are needed to statistically power the sensitivity for the test by identifying at least 100 Down syndrome cases. Sequenom is also developing tests for chromosomes 18, 13, X, and Y, which larger studies can also help advance.

To be clear, this 3-5000 sample launch-enabling validation study should be completed by June 2009 and will enable Sequenom's CLIA lab to offer the LDT test in compliance with all testing regulations. These results are expected to also meet the level of evidence expected by many leaders in the Obstetrics community and therefore drive at least early-adoption if not broad utilization. A larger study called *RNA* is also being conducted, the results of which would be expected to appear in a high quality peer-reviewed journal shortly after the launch of the test. The RNA study would be the kind of study that one might expect more conservative physicians to wait for before offering the test to their patients.

The RNA Noninvasive Aneuploidies (RNA) Study: A Prospective Study to Validate the Sensitivity and Specificity of the SEQureDx T21 Laboratory Developed Test

What is the RNA Study?

The study described above is for the in-house validation of the SEQureDx Technology as an LDT test at Sequenom's CLIA laboratory. In addition, a parallel, multi-center observational trial will be initiated to independently determine and report on the SEQureDx Technology test performance. The ***R***NA ***N***oninvasive ***A***neuploidies (***RNA***) **Study** is an independent study, albeit funded by Sequenom. The design, conduct, data collection, data analysis, interpretation, and reporting of results will be the responsibility of the Principal Investigators, outside of Sequenom's direct control. The study will be directed by Dr. Jacob Canick and Dr. Glenn Palomaki of Women & Infants Hospital of Rhode Island (affiliated with Alpert Medical School of Brown University). Both

investigators have been involved in large-scale clinical trials in the area of prenatal screening and have published extensively on that subject.

The ***RNA* Study** is an observational trial whose primary aim is to document and publish in a high quality peer-reviewed journal the performance (clinical sensitivity and false-positive rate) of a laboratory developed test (LDT) using fetal RNA in maternal plasma to identify Down syndrome in early pregnancy. The secondary aim is to develop a sample bank to allow documentation of subsequent improvements in the LDT, including increased proportion of interpretable samples (coverage) and the identification of other aneuploidies (e.g., Trisomy 18).

The ***RNA* study** will evaluate test performance by obtaining blood samples from up to 10,000 pregnant women prior to their having either early 2nd trimester amniocentesis or late 1st trimester CVS. Approximately 100 samples from women with a Down syndrome pregnancy are expected to show up in the first trimester cohort and another 100 samples from Down syndrome pregnancies in the second trimester cohort. Clinical performance of the test will be examined as a function of laboratory testing site (three sites), gestational age (late first and early second trimester), and maternal race/ethnicity (e.g., Caucasian, Asian, and African American). The LDT methodology used in this study (sample collection, shipping and testing) will represent, as closely as possible, a methodology that could be routinely offered as a clinical test.

Why launch the SEQureDx T21 test early next year given that you will not yet have completed the very large RNA Study?

Although upwards of 3-5,000 study samples will have been analyzed and reported at the time of the LDT launch by June 2009, the potential clinical practice changing nature of this test also calls for a study designed, conducted, and analyzed by independent scientists and clinicians.

Ethical considerations

Invasive procedures such as chorionic villus sampling (CVS) or amniocentesis place the fetus at unnecessary risk. In fact, studies have shown that the risk of miscarriage is approximately 1:100 to 1:300 depending on the invasive procedures employed. In Sequenom's validation study, the number of Trisomy 21 positives is expected to be about 75-150 in a total population of up to 5,000 women while the RNA study is expected to total 200 positive cases out of 10,000 women sampled. If a low prevalence population study were conducted, the total number of patients that would need to be evaluated to find 200 positives would exceed 100,000 patients.

Down syndrome is relatively uncommon, with a prevalence of about 1:733 births. To meet the target of 300-350 cases, approximately 200,000 women would need to be tested in both studies. Although this would be considerably more expensive, that is not the main reason this study design should not be employed. First, it would not be possible to karyotype all 200,000 women because of the associated fetal risk (more than 1300 normal fetuses might be spontaneously lost/miscarried). This makes the true identification of all Down syndrome and unaffected

pregnancies impossible without identifying the outcome of every birth and miscarriage. Needing consent and compliance from 200,000 women would require enrolling far more because of drop-outs. Such a design may seem appropriate, but it is not.

Many screening paradigms have been introduced to the market and not one has employed a primary validation strategy of enrolling patients without enriching for higher-risk women, for all the ethical and practical considerations described above. The RNA study protocol has been developed by clinical and academic leaders in this field to be entirely consistent with how such tests are typically introduced into clinical practice.

Regulatory Considerations

The U.S. Food and Drug Administration (FDA) have repeatedly stated that they intend to maintain enforcement discretion with regard to LDTs and CLIA laboratories. Under the Clinical Laboratory Improvement Amendments (CLIA in 1988), clinical laboratories are allowed to develop Laboratory Developed Tests and may use them for in vitro diagnostic use following validation. On August 7, 2008, FDA sent the lab conducting OvaSure™ testing a public letter that informed the laboratory that the OvaSure™ test, in the FDA's estimation, was a high risk test that has not received adequate clinical validation and may harm the public health. FDA invited the lab to discuss the validation with them.

The lab's evidence for validation came from a paper in Clinical Cancer Research (Visintin, I, etal, Clin Cancer Res. 2008, Feb 15:14(4): 1065-72). FDA noted that the study was carried out on two biased patient populations, healthy normals and women who already experienced ovarian cancer. FDA stated that the data presented in this paper were insufficient to establish performance characteristics of a test in high risk women who might have ovarian cancer because such women were not properly and prospectively represented in the population tested. The FDA appeared to consider the OvaSure™ test study design biased because samples were pre-selected retrospectively from two study populations: healthy/normal vs. those who already experienced ovarian cancer.

This example has been mentioned at times as evidence that FDA might want to impose strict regulation of all CLIA tests, yet the poor design characteristics of the OvaSure validation study make it a non-comparable for SEQureDx and other LTD tests where study design is of higher quality and meets with FDA guidelines.

Differences between OvaSure™ and Sequenom's SEQureDx Trisomy 21 Study Design:

The OvaSure™ test study differs significantly from the ones that will be run to validate Sequenom's SEQureDx Technology. Key areas of differentiation include prospective *vs.* retrospective study

design, selection of samples with known outcome, sample size, and appropriateness of the study population.

Sequenom's SEQureDx T21 study is a large prospectively collected study where the patient outcome is not known and the study was appropriately designed and statistically powered to answer a specific question: is an LDT test acceptable for the detection of Down syndrome in early pregnancy. The Sequenom study design evaluates the real world laboratory experience rather than a weakly simulated one.

FDA issued a guidance document titled: *Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests:* dated March 13, 2007. In this document, FDA states that the estimates for diagnostic accuracy are subject to spectrum bias when the subjects included in the study do not include the complete spectrum of patient characteristics, i.e. spectrum bias exists if one fails to enroll subject/specimens across the entire range of disease state by omitting intermediate cases, which may be more difficult case to diagnose. By selecting only healthy/normal vs. women who experienced ovarian cancer, the investigator biased the OvaSure™ study by eliminating an important potential study parameter (difficult-to-diagnose intermediate cases).

The Sequenom prospective study eliminates the potential for bias and the problem is further simplified by the fact that Down syndrome is a binary genetic state, not a progressive disorder; therefore, there are no intermediate cases. Furthermore, FDA understands and makes provisions for rare conditions such as Down syndrome (1:733) by allowing for the enrichment of higher risk patients using known risk diagnostic correlates.

Would the LDT version of the RNA Study test qualify as an IVDMIA?

No. The new IVDMIA guidance (2007) specifically excludes prenatal Down syndrome tests such as the triple tests because physicians could interpret the results themselves due to extensive training and experience. Although the execution of the RNA test is complex, interpretation is not. In the Analyst Day presentations, Dr. Elizabeth Dragon showed the raw mass spectroscopy data for normal, Down syndrome, and no-call samples, all of which were clearly distinct from one another and could be read by anyone with minimal instruction. In fact, it is similar in interpretation to the karyotype, which is considered a routine test in obstetrics. Thus, the method of risk calculation is not a "black box", and therefore not covered by IVDMIA draft guidance.

Is the proposed Validation Study design subject to spectrum bias?

In the FDA letter to the laboratory conducting the **OvaSure™ test**, spectrum bias was the basis for concluding that insufficient data were available to determine **OvaSure™** clinical performance. Spectrum bias can occur in the study of a diagnostic test when one (or more) of the subgroups is not representative (e.g., comparing healthy individuals to ones with advanced disease without intermediate patients). Unlike cancer, the genetic basis of Down syndrome does not change over

time. Three 21 chromosomes remains the hallmark diagnosis from conception to adulthood. Since the RNA test is based on identifying the presence or absence of three 21 chromosomes, it is not subject to spectrum bias.

What sort of study designs have the FDA accepted in the past for similar claims.
FDA has not approved any class III devices for Down syndrome testing. Historically, the FDA has allowed PMAs to be performed using stored samples from several thousand unaffected pregnancies interspersed with known affected pregnancies. For example, several successful PMAs were approved for maternal serum AFP testing as an aid in the diagnosis of open neural tube defects. These involved three geographically disparate sites identifying 3000 serum samples from women with unaffected pregnancies (at an appropriate period of gestation) along with similar samples from 10 to 30 women with affected pregnancies. These stored samples were then run in a 'simulation' of a high risk cohort. Open neural tube defects occur in about 1:2000 pregnancies. The high risk cohort had a prevalence of about 1:100; a 20 fold increase. This was considered a least burdensome approach.

Based on current guidelines and precedents, there is no reason to believe that FDA would need to regulate SEQureDx under PMA guidelines on the basis that Sequenom's study design is deficient. By comparison to the AFP study and other study designs accepted by the FDA in the past, it is Sequenom's position that they are conducting an ethical and appropriately designed/powered trial to validate the SEQureDx Trisomy 21 LDT test for the detection of Down syndrome in early pregnancy.

Safe Harbor: Except for the historical information set forth herein, statements herein including but not limited to statements relating to, expectations, opinions, or beliefs regarding government regulation, forthcoming products and applications, the potential for the generation of certain volumes and types of results using various products and applications, future diagnostic product development, particularly non-invasive prenatal diagnostics, and any statements relating to the Company's goals, plans, missions, expectations, beliefs, and the like, are forward-looking statements within the meaning of the "safe harbor" provisions of the Private Securities Litigation Reform Act of 1995. These forward-looking statements are subject to risks and uncertainties that may cause actual results to differ materially, including the risks and uncertainties inherent in introducing new products and new product applications, product development and commercialization, particularly for molecular diagnostics products and services applications, including non-invasive prenatal diagnostic products and services, the risks and uncertainties associated with government regulation, SEQUENOM's technologies and those of its competitors, and other risks detailed from time to time in SEQUENOM's SEC reports, including SEQUENOM's most recently filed Annual Report on Form 10-K and its most recently filed periodic report. These forward-looking statements speak only as of the date hereof. SEQUENOM disclaims any intent or obligation to update these forward-looking statements.