

# Technical standards and guidelines: Prenatal screening for Down syndrome

This new section on “Prenatal Screening for Down Syndrome,” together with the new section on “Prenatal Screening for Open Neural Tube Defects,” replaces the previous Section H of the American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories\*

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**Key Words:** Down syndrome, maternal serum screening, prenatal diagnosis, genetic screening

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**Disclaimer:** These standards and guidelines are designed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to these standards and guidelines does not necessarily ensure a successful medical outcome. These standards and guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical molecular geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the laboratory record the rationale for any significant deviation from these standards and guidelines.

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This statement is intended to augment the current general American College of Medical Genetics (ACMG) Standards and Guidelines for Clinical Genetics Laboratories and to address validation guidelines specific to second trimester maternal serum screening for Down syndrome. Individual laboratories are responsible for meeting the CLIA/CAP quality assurance standards with respect to appropriate sample documentation, assay validation, general proficiency, and quality control measures.

## DS2 BACKGROUND ON DOWN SYNDROME

**DS2.1 OMIM NUMBER.** 190685, Down syndrome.

**DS2.2 BRIEF CLINICAL DESCRIPTION.** Down syndrome (trisomy 21) is one of the most common genetic causes of moderate to severe mental retardation. Virtually all individuals with Down syndrome are hypotonic and have minor dysmorphic features, which can include up-slanting palpebral fissures, epicanthal folds, flat nasal bridge, Brushfield spots of the iris, shortened fifth finger, and transverse palmar crease. Congenital heart disease is present in 40%, and 5% have gastrointestinal anomalies such as duodenal atresia or Hirschsprung disease. The incidence of childhood leukemia is increased up to 20 times over

that of the general population. Adults with Down syndrome experience neuronal degeneration identical to that present in Alzheimer's disease. Down syndrome individuals without congenital heart disease can live beyond 60 years of age.

**DS2.3 ETIOLOGY.** Down syndrome is caused by the presence of an extra copy of chromosome 21, either as a free chromosome, a Robertsonian translocation, or a reciprocal translocation involving chromosome 21. Approximately 95% of cases result from sporadic nondisjunction during parental meiosis. The nondisjunction is maternal in 95% of cases, and 77% of the maternal nondisjunction occurs during meiosis I. The risk of having a child with Down syndrome increases with advancing maternal age. Down syndrome can be inherited when one parent carries a translocation involving chromosome 21. If a parent carries a Robertsonian translocation, the risk to the offspring is dependent on the sex of the carrier parent.

**DS2.4 LABORATORY DIRECTOR.** Although the prenatal screening laboratory utilizes clinical chemistry methods such as enzyme immunoassays, its function differs, because the results require a unique kind of interpretation. This interpretation puts the results of the test into the appropriate context of a priori risks as determined by maternal age, gestational age, and family history. The laboratory director is often called upon to provide consultation regarding these risks and options for further action. To address these unique requirements, the laboratory director should meet the standards set out in Section B3 of the ACMG guidelines. When prenatal screening for Down syndrome is performed in a clinical chemistry laboratory in which the director does not meet these standards, the laboratory should have a demonstrated relationship with an individual

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who does meet the standards set out in Section B3. That person should be available to aid in interpretation and provide consultation when requested.

**DS2.5 SCREENING VERSUS DIAGNOSTIC TESTING.** Prenatal testing for Down syndrome by measurement of multiple second trimester maternal serum markers is considered a screening test. Asking a woman her age and offering amniocentesis if she is age 35 or older is also a screening test. The diagnostic test for Down syndrome is the karyotyping of fetal cells, usually obtained by amniocentesis (chorionic villus sampling may also be used). The distinction between a screening and a diagnostic test is important, because the goals and expectations differ for clinical sensitivity and specificity, costs, and acceptable level of invasiveness. The interpretation of maternal serum markers is not diagnostic of any condition. Rather, the screening process identifies pregnancies that are at sufficient risk for Down syndrome to warrant genetic counseling and the offer of additional testing, such as amniocentesis and karyotyping. The clinical sensitivity and specificity of this screening test will be a function of several factors, including maternal age, number of analytes measured, assay precision, the method of gestational dating, and the risk cut-off level used to determine “screen positive” results.

### DS3 SECOND TRIMESTER MATERNAL SERUM SCREENING FOR DOWN SYNDROME

Prenatal screening for Down syndrome is best implemented in the context of a comprehensive program that coordinates preanalytic, analytic, and postanalytic components of the process. These Standards and Guidelines will focus on Down syndrome risk assessments that combine maternal age-associated risk with interpretations of the levels of second trimester maternal serum screening markers. These markers include  $\alpha$ -fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and dimeric inhibin-A (DIA).<sup>1-7</sup> Unless otherwise noted, measurements of the free  $\beta$  subunit of hCG can be used interchangeably with hCG measurements.<sup>8</sup>

#### DS3.1 PATIENT AND PROVIDER INFORMATION.

**DS3.1.1 Patient information.** Laboratories should either provide educational materials (e.g., brochures, videotape) for use by patients in consultation with their providers or, at a minimum, provide information about where such materials can be obtained. Many laboratories and professional organizations (e.g., ACOG, National Society of Genetic Counselors, regional genetics groups) have produced, and in some cases formally evaluated, materials that are in effective formats, at appropriate reading levels, and available in multiple languages. These materials provide general information about the disorder, test performance, patient rights, eligibility, test interpretation, treatment options, costs, risks and benefits of testing, and what to expect if the screening test is positive.

**DS3.1.2 Informational materials for health care providers.** Laboratories should supply providers with informational materials that include the following:

**DS3.1.2 (a)** The sampling process and how samples should be labeled and transported.

**DS3.1.2 (b)** Samples of test requisitions that must accompany samples to provide information needed for identification and accurate test interpretation.

**DS3.1.2 (c)** General information on testing, such as laboratory turn-around time and whether results will be telephoned/faxed, mailed, or electronic transmission.

**DS3.1.2 (d)** Information about expectations for clinical test performance (sensitivity, specificity and failure rate) and reporting formats.

**DS3.1.3 Informed consent.** Patients should be informed about the benefits and limitations of prenatal screening before testing. It is the duty of the health care professional, not the laboratory, to inform and obtain consent for testing, but the laboratory sometimes is required to document such consent (e.g., in New York state).<sup>9</sup> It is the laboratory’s responsibility to provide sufficient information about prenatal screening to the health care provider to ensure that an appropriate specimen is obtained and to facilitate educating the patient and obtaining consent.

**DS3.1.4 Requisition forms and intake information.** For the most reliable interpretation, laboratories should have a mechanism to collect pretest clinical information that includes the following:

**DS3.1.4 (a)** Basic required demographic information (see C2.4 and C3);

**DS3.1.4 (b)** Gestational age (see DS3.5.3.1 to DS3.5.3.4);

**DS3.1.4 (c)** Maternal weight;

**DS3.1.4 (d)** Maternal race (at least Caucasian and Black/African American);

**DS3.1.4 (e)** Presence of maternal insulin dependent diabetes mellitus prior to pregnancy;

**DS3.1.4 (f)** Number of fetuses;

**DS3.1.4 (g)** Previous screening in the current pregnancy (e.g., initial or repeat serum sample);

**DS3.1.4 (h)** Family history of Down syndrome;

**DS3.1.4 (i)** Whether a successful diagnostic test has already been performed (e.g., CVS).

The laboratory may choose to contact the provider if critical patient information does not accompany the specimen. If the

laboratory does not obtain this information, the written report should indicate that the information is missing and what information, if any, was used in the interpretation. In some cases, including information on the report about the potential impact of the missing information may be warranted (e.g., maternal weight and race); in other cases, full interpretation may not be possible (e.g., no maternal age or no gestational age).

### **DS3.2 SPECIMEN COLLECTION AND TRANSPORTATION.**

**DS3.2.1 Specimen collection.** Blood samples should be collected using standard phlebotomy techniques. The laboratory should specify what samples are acceptable (e.g., whole blood, serum separator tube, centrifuged serum separator tube). Specimen containers should be labeled with the patient's name and draw date.

**DS3.2.2 Specimen transportation.** Acceptable specimen handling from collection site to the laboratory should be specified, including packaging, mode of transportation (e.g., courier, United States mail, overnight transport), and temperature range (sample stability is discussed in Section DS3.3.3).

### **DS3.3 SPECIMEN PROCESSING AND STORAGE.**

**DS3.3.1 Criteria for sample rejection.** Variables that can affect the acceptability of a sample for Down syndrome screening should be established by the laboratory, and may include both clinical (e.g., gestational age out of range) and sample-related characteristics (e.g., inappropriate sample type, insufficient quantity, gross hemolysis). See also Sections C2.4 to 2.6.

**DS3.3.2 Specimen processing.** Protocols should be designed to avoid contamination, tampering, or substitution. Handling samples must be in accordance with OSHA guidelines, with the express understanding that any human fluids may harbor infectious agents.<sup>10</sup>

**DS3.3.3 Sample stability.** AFP, hCG, and DIA can be reliably determined in sera stored at 4° to 8°C for days and at -20°C for years. uE3 is not stable in whole blood; samples should be promptly centrifuged in separator tubes or separated from the clot. uE3 is stable in sera stored at 4° to 8°C for days. In the past, some kits produced systematically different uE3 values after the sera were frozen and thawed. If frozen samples are to be used to derive medians, possible freeze/thaw effects should be examined. For optimal performance, shipping time should be minimized (e.g., express mail, courier service), and samples should not be exposed to high temperatures. Free  $\beta$  subunit is not stable in serum when exposed to high temperatures (e.g., daytime summer temperatures in the southern United States), due to dissociation of intact hCG. If free  $\beta$  is to be measured, samples must be protected from high temperatures (e.g., cool packs with overnight shipment in the summertime). Shipping samples in the form of blood spots can also result in improved stability. Also see Sections C2.4 to 2.6.

**DS3.3.4 Establishment of laboratory policies regarding specimen retention.** See Sections C2.7 to 2.8 for more information.

### **DS3.4 ASSAY METHODOLOGIES.**

**DS3.4.1 Detailed analytic procedures.** Guidance on developing assay protocols is available. See C5, C6, and Validation C8.3.

**DS3.4.2 Methodology and reagents.** In the United States, the Food and Drug Administration (FDA) has not licensed any kits for Down syndrome screening. All have Class II approvals (510K) for other clinical applications, which restrict manufacturers from making clinical claims about Down syndrome. Published guidelines from NCCLS provide procedures for evaluating manufactured kits, including precision, linearity, stated performance characteristics, and guidelines on clinical sensitivity and specificity.<sup>11</sup> Available AFP kits (licensed by FDA as an aid in diagnosing open neural tube defects) include immunometric and radioimmunoassay methods. All appear capable of measuring AFP reliably in the range of values important for Down syndrome screening (10 to 50 IU/mL).<sup>12</sup> Kits for measuring uE3 and hCG are also available from several manufacturers. Fewer options are available for purchasing kits for measuring DIA and free  $\beta$  hCG due to patent/licensing agreements. The kit used for DIA must target levels important for Down syndrome screening (10 to 1500 pg/mL).

**DS3.4.3 Standards and calibration procedures.** AFP standards can be calibrated in either mass units (ng/mL) or international units (IU/mL). Each AFP kit manufacturer provides a factor for converting mass units into international units; conversion factors should be considered manufacturer-specific. Most hCG kits are calibrated in mIU/mL using the 1st International Reference Preparation (equivalent to the 3rd International Standard). Results can be reported as IU/mL (e.g., 3.2) or mIU/mL (e.g., 3200). DIA assay results are usually reported in pg/mL. Free  $\beta$  hCG kits can be calibrated in mIU/mL or ng/mL. Commercially available kits provide calibrators and specific calibration protocols. Laboratories utilizing "home-brew" assays or modifying kit assay protocols are responsible for determining calibration protocols and validating performance.

### **DS3.4.4 Preparation, characterization, and use of controls.**

**DS3.4.4.1 Assay controls.** Commercial uE3 controls might need to be diluted with nonpregnancy serum to achieve concentrations appropriate for levels found in the second trimester. In-house pooled controls, commercially available controls, or controls received in kits serve as checks on reagents and technical performance. Advantages of in-house pooled controls include a sample matrix that more closely resembles patient samples, levels specifically targeted for Down syndrome clinical action points (e.g., lower AFP and higher hCG levels), and control lots prepared with long expiration dating to aid in assessment of kit master reagent lot changes and long-term

assay drift. An alternative for long-term monitoring is commercial controls bought in sufficient quantity to last a year or more. No commercial controls are available for DIA.

**DS3.4.4.2 Repeat assay controls.** Repeat assay controls (RACs) can also be helpful in monitoring performance variability. To assess short-term performance, unfrozen patient samples are chosen at random from recent assays and re-assayed to monitor intra- and interassay precision. Because the serum analytes currently in wide use are essentially stable when frozen and thawed (see DS3.3.3), reassaying stored patient samples from the time period when the current median values were established can also help to identify long-term drift and determine whether reference data need to be updated.

**DS3.4.4.3 Concentration of controls.** Each assay should contain at least two quality control samples that fall at clinical action points (three controls may be required to comply with some licensure requirements). For example, an hCG high control could be targeted at a value equivalent to 2.0 or 2.5 multiples of the median value (MoM) at 16 weeks, along with a second mid control near the median (1.0 MoM).

**DS3.4.4.4 Characterization of control materials.** After preparation and aliquoting, performance ranges for in-house pooled controls can be set using standard clinical laboratory quality control approaches. Controls received with licensed kits have an acceptable target range specified by the manufacturers, but laboratories may wish to establish an in-house range. This information is used to accept or reject individual control results or a whole assay, so care should be taken to set appropriate ranges and avoid unnecessary result ejection.

#### DS3.4.5 Quality control (QC).

**DS3.4.5.1 Type and frequency of QC assessments.** Standard approaches used in the clinical laboratory are appropriate for internal QC of these assays.

**DS3.4.5.2 Measures of repeatability both within and between runs.** As part of the initial method validation, the laboratory should demonstrate that intra- and interassay variation reported by the manufacturer can be reproduced.

**DS3.4.5.3 Routine equipment calibration and preventive maintenance.** Standard approaches used in the clinical laboratory are appropriate. In many cases, calibration and maintenance protocols are set by the product/equipment manufacturer (see DS3.4.4.4).

### DS3.5 ASSAY RESULTS.

**DS3.5.1 Converting assay results to multiples of the median.** In order for each of the analytical measurements to be interpreted, each result in mass or international units must first be converted to a MoM for a given gestational age. The resulting MoM levels can then be adjusted for other factors such as maternal weight.

**DS3.5.2 Normative data.** It has been established that values obtained from different lots from the same manufacturer or from different manufacturers may demonstrate systematic bias. Therefore, it is essential that each laboratory establish its own normative data or, at a minimum, demonstrate that data obtained from another source are appropriate for its screened population.

**DS3.5.2.1 Source of medians.** Package insert (commercial) medians should not be used, even for a short time. Several methods exist that can be utilized to establish reliable medians.

**DS3.5.2.2 Sample size.** Ideally, 100 samples for each gestational week from 15 through 20 would be used to calculate median values. Because these analytes are stable, it is possible to use stored frozen specimens collected over several years. A consecutive series of samples is appropriate; it is not necessary that all samples be from unaffected singleton pregnancies. Using regression analysis allows use of fewer samples (e.g., 300 spread over the 15- to 20-week period) to establish reasonable medians.<sup>13</sup>

**DS3.5.2.3 Computing medians.** “Smoothing” the observed median values by weighted regression analysis provides reliable and accurate medians. Appropriate models for each of the analytes can be found in the literature (AFP and uE3 fit a logarithmic linear model, hCG fits an exponential model, and DIA fits a quadratic model). These methods also allow median values to be extrapolated for weeks in which little data are available. Using median values that are specific to each day of gestation will further improve screening performance.

**DS3.5.2.4 Expected change in medians by gestation.** Maternal serum AFP and uE3 levels increase by a constant percentage per week in the second trimester (approximately 10% to 15% for AFP and 20% to 25% for uE3). Levels of hCG and free  $\beta$  hCG decrease by about 25% from 15 to 17 weeks, but decrease from 18 to 20 weeks at a much lower rate. Measurements of DIA are lowest at about 17 weeks; the 15- and 19-week medians are about 10% higher.

#### DS3.5.3 Variables that have significant impact on calculation of the MoM level.

**DS3.5.3.1 Time of testing.** Clinical sensitivity and specificity for Down syndrome screening are essentially constant between 15 and 20 weeks' gestation. However, the optimal time for screening is set at 16 to 18 weeks because of the concurrent use of AFP measurements to screen for open neural tube defects. If samples are collected for Down syndrome screening at 14 weeks' gestation, the significantly poorer screening performance for open neural tube defects should be noted in the laboratory's report. Under special circumstances, laboratories may accept samples later than 20 weeks' gestation with the understanding that clinical options may be limited.

**DS3.5.3.2 Gestational age.** Gestational age may be expressed in completed weeks (15 weeks, 5 days is 15 completed weeks). Expressing results in rounded weeks (15 weeks, 5 days is 16 completed weeks) is not recommended. Screening performance is improved by expressing gestational age as weeks and days or decimal weeks (15 weeks, 5 days is 15.7 weeks).

**DS3.5.3.3 Dating method to use.** The most common method for determining gestational age is dating by the first day of the last menstrual period (LMP). Ultrasound measurement of crown-rump length (CRL) in early pregnancy provides an accurate estimate of gestational age to within 7 days. In the second trimester, ultrasound dating is accurate to within 10 days, but some measurements (e.g., shortened femur and humeral length) are associated with the presence of Down syndrome, and their use (alone or in combination with other ultrasound measurements) will reduce screening performance. Biparietal diameter (BPD) measurements are not altered in the presence of Down syndrome. This measurement is also the recommended dating method for use in screening for open neural tube defects (see ONTD3.5.3.3). If available, ultrasound dating using CRL or BPD improves both the sensitivity and specificity of screening compared to LMP dating.

**DS3.5.3.4 Incorporating dating method.** The method of determining gestational age can be taken into account in two ways when providing Down syndrome interpretations. First, separate medians can be calculated for those pregnancies dated by LMP and those dated by ultrasound measurements. Secondly, separate population parameters can be utilized in determining Down syndrome risk (see DS3.5.7.5).

**DS3.5.4 Factors that may be used to adjust the MoM levels.** The following are interpretive refinements based on patient demographics and other pregnancy-related information that are less critical than taking gestational age into account, but will improve screening performance by optimizing the interpretation. Currently, most laboratories take the following factors into account.

**DS3.5.4.1 Maternal weight.** For each of the analytes, levels are on average higher in lighter weight women and lower in heavier weight women. Adjusting for maternal weight provides only minimal improvement in Down syndrome screening.<sup>14</sup> However, weight adjustments should be performed for other reasons. AFP levels should be adjusted when screening for open neural tube defects, and AFP, uE3, and hCG levels should be adjusted when screening for trisomy 18. Laboratories should utilize published weight adjustment formulas only until in-house data are collected and new laboratory-specific formulas derived.

**DS3.5.4.2 Maternal race.** Correction for maternal race should be made for AFP and possibly hCG measurements. For both analytes, levels in Black/African American women are about 10% to 15% higher than in Caucasian women.<sup>12</sup> If suf-

ficient data are available, the preferred adjustment method is to calculate a separate set of medians for each of the racial groups. If too few observations are available in one of the groups, a correction factor can be applied to the MoM when screening those pregnancies.

**DS3.5.4.3 Maternal insulin-dependent diabetes mellitus (IDDM).** Correction for maternal IDDM should be made for AFP. Most programs take this into account by using an adjustment factor for AFP MoM levels. Other analytes are less influenced, but the laboratory director may want to adjust these as well by factors available in the literature.<sup>12</sup> There is no consensus on whether this correction should be applied to women with gestational diabetes.

**DS3.5.4.4 Use of multiple correction factors to calculate the MoM.** Calculating MoM results for a 200-pound Black/African American woman with IDDM would require at least three adjustments to the AFP MoM. Although data are sparse, programs can make the assumption that the effects are independent. Although most data are derived from studies of mainly Caucasian women, the assumption is usually made that a similar effect will be seen in Black/African American women.

**DS3.5.5 Prenatal screening software for computing and reporting patient-specific risk for Down syndrome.** Laboratories must be able to compute risks for Down syndrome. The use of specialized software applications is generally considered a necessity for Down syndrome screening due to the complex nature of calculating and interpreting the results, the need for patient-specific interpretive reports, and the large number of samples processed.

**DS3.5.5.1 Down syndrome risks.** Patient-specific Down syndrome risks are generated by complex computer algorithms that are integral to prenatal screening. Such software applications can be obtained commercially or developed in-house and must be verified prior to routine clinical use.

**DS3.5.5.2 Risk algorithm.** The commonly used algorithm to assign a patient-specific risk utilizes the MoM results (adjusted for variables such as weight and race, as discussed earlier) to calculate a likelihood ratio based on the overlapping multivariate Gaussian distributions defined by the affected and unaffected distribution parameters. The a priori risk for Down syndrome, based on maternal age, is then multiplied by the corresponding likelihood ratio to generate the patient-specific risks. Equations to compute the a priori risk for a given maternal age have been published.

**DS3.5.5.3 Population parameters.** Risk algorithms utilize published or in-house population parameters for each of the analytes, expressed as log means and log standard deviations for unaffected pregnancies and pregnancies affected with Down syndrome.<sup>2,8,12</sup> In addition, pairwise correlation coefficients in both case and control pregnancies and truncation limits are needed to generate reliable risks. These population

parameters can vary, based on gestational dating method (see DS3.5.7.5).

**DS3.5.5.4 Combinations of factors.** There is no formal consensus on which adjustments to the result or a priori risk to include in an interpretive software program. Specifically how to include them, or whether to include them, is a decision left to the laboratory director.

**DS3.5.6 Selection of screening cut-off levels.** Definition of screen positive and negative results: Screening for Down syndrome relies on the patient-specific risk as the screening variable. This risk is computed by multiplying the age-associated a priori risk times the likelihood ratio derived from the multiple analyte measurements.

**DS3.5.6.1 Down syndrome risks.** Down syndrome risks can be expressed as risks in the second trimester or at term. If the term risk for an average 35-year-old woman is reported to be 1:350, the corresponding (and equivalent) second trimester risk is  $1:350 \times 0.77$  or 1:270. The factor of 0.77 represents a reliable estimate of the proportion of Down syndrome fetuses that survive from the early second trimester to term. Term Down syndrome risks can be computed for maternal age in completed years or in decimal years. Published equations can be utilized to compute these risks.<sup>15,16</sup>

**DS3.5.6.2 Prior risk.** Historically, women have been offered diagnostic testing if they were 35 years of age or older (term risk of about 1:350; second trimester risk of about 1:270). Screening programs that combine AFP, uE3, and hCG measurements with maternal age may continue to use this cut-off level with the expectation of about a 70% Down syndrome detection rate and a 7% false-positive rate. It is also acceptable to choose a higher risk cut-off level (e.g., term risk of 1:250) that is associated with a lower detection rate of about 65% and a lower false-positive rate of about 5%. Detection and associated false-positive rates vary not only by screening cut-off level chosen, but also by the age distribution of the women tested, the number of markers chosen, and the method of gestational dating.

**DS3.5.7 Variables that impact either the prior risk or the screening parameters.**

**DS3.5.7.1 Time of testing.** The optimal time in the second trimester for Down syndrome screening is 16 to 18 completed weeks. Although reliable interpretation of Down syndrome risk might be possible at 14 and 15 weeks' gestation, this practice is discouraged because of the associated poor open spina bifida screening performance (see ONTD3.5.7.1) Although reliable risks might be possible after 20 weeks' gestation, screening this late in pregnancy limits the options available should a positive test result be found.

**DS3.5.7.2 Multiple gestation.** Down syndrome screening in twin pregnancies is less effective than for singleton pregnancies. Algorithms for assigning a "pseudorisk" have been pub-

lished.<sup>12,17,18</sup> Whether or not a program interprets samples from known twin pregnancies is left to the laboratory director. Pseudorisks do not have to be reported. However, when pseudorisks for twins are reported, the limitations should be mentioned in the report.

**DS3.5.7.3 Repeat testing.** Obtaining a second specimen for repeat testing is discouraged as part of Down syndrome screening (one exception is when sample mix-up is suspected). If an initial sample is later found to have been collected too early, the subsequent sample is considered the first interpretable sample. Should a known repeat sample be submitted to the laboratory, it should not be interpreted as though it were an initial specimen.<sup>19</sup> Published methods exist to combine the information from the two samples in order to provide a reliable interpretation.<sup>20,21</sup> Other methods of interpretation may be acceptable.

**DS3.5.7.4 Family history.** Family history of Down syndrome increases the a priori risk, depending on the degree of relatedness and the mode of inheritance. Laboratories may choose to include a recommendation for genetic counseling in the report if a family history suggests an a priori risk higher than the patient's age-related risk. Alternatively, laboratories may choose to incorporate a previous affected pregnancy into the Down syndrome risk estimate using published algorithms.

**DS3.5.7.5 Method of assigning gestational age.** Ultrasound dating has the effect of "tightening up" the distribution for each of the analytes in both unaffected and affected pregnancies. For this reason, separate sets of distribution parameters can be used for LMP and ultrasound dated pregnancies. Although ultrasound usually generates a reliable estimate of gestational age in unaffected pregnancies, several measurements (humeral and femur bone) are known to be smaller in Down syndrome pregnancies. Optimally, BPD measurements should be used as these represent unbiased estimates of gestational age in Down syndrome pregnancies. This has the added advantage of improved screening performance for open neural tube defects (see ONTD3.5.3.3). First trimester CRL measurements also provide an unbiased estimate of gestational age but do not improve screening for open neural tube defects. Gestational age based on composite second trimester measurements is often the only estimate available and can be used in place of dating by LMP.

**DS3.5.8 Technical limitations of the methodology for the intended use.** Laboratories need to select reagents for maternal serum screening to meet performance requirements that are more stringent than for other intended uses. Kits need to be both precise and relatively accurate (different kits need not give identical values on the same sample provided in-house reference data are established using the same kit). Coefficients of variation (CVs) of 10% or greater can adversely impact both detection and screen positive rates.<sup>22</sup> It is also important that kits/reagents are stable over a long period of time, and that lot-to-lot variability is minimized.

### **DS3.5.9 Long-term assessment of variability and performance.**

**DS3.5.9.1 Assay controls.** In-house pooled controls (or commercial products obtained in sufficient quantity to last a year or more) and repeat assay controls (RACs) are valuable for monitoring long-term assay drift and lot-to-lot variability (see DS3.4.4.1–DS3.4.4.4).

**DS3.5.9.2 Normative data review.** Median values should be reviewed at regular intervals by the laboratory and recalculated when necessary, at least annually. Medians should be recalculated if there is a shift in analyte values  $> 10\%$  or a shift between 5% and 10% that is consistent over time, whether due to observed assay drift or reagent lot change. Shifts in analyte values can be monitored by computing the overall median MoM level (see DS3.5.9.4). Observations from samples tested in the previous months or years should be used only if epidemiological monitoring shows the median MoM has been stable. Alternative methods of revising medians may be necessary if a significant shift has been observed (see DS3.5.2).

**DS3.5.9.3 Evaluating medians with new reagent lots.** Between 25 and 50 patient samples and current controls can be run on the old and new kit/reagent lot and the relationship between the two examined using techniques of regression analysis and method comparison. That relationship can then be applied to the existing medians to derive temporary new medians that can be used until sufficient data are available from the new lot for the optimum analysis (see DS3.5.2.2).

**DS3.5.9.4 Epidemiological monitoring.** In order to monitor assay and program performance and to identify possible areas of concern, screening programs must perform epidemiological monitoring.<sup>23</sup> Such monitoring, at a minimum, should include the periodic computation (monthly or weekly depending on numbers of samples processed) of the median MoM for each of the analytes and the determination of the statistical significance of any deviation from 1.00. Any corrective action needs to be documented. Laboratories should periodically compute their initial positive rate and compare it to expected published rates, after taking into account variables such as the number of analytes, screening cut-off level used, and the proportion of pregnancies dated by ultrasound.

**DS3.5.10 Long-term monitoring.** In recent years, stricter privacy and confidentiality policies and in some cases laws have made it much more difficult to collect pregnancy outcome information and even information regarding follow-up of medical procedures (such as ultrasound and amniocentesis) performed subsequent to positive screens. If possible, laboratories should collect pregnancy outcome information on the women with initial screen positive results. This information might include the proportion of pregnancies reclassified as screen negative, the diagnostic testing uptake rate, and the number of affected pregnancies identified either in the second

trimester or at term. For those laboratories that have sufficient resources, complete pregnancy follow-up is recommended and will allow the determination of the Down syndrome detection rates. An alternative approach acknowledged by some regulatory agencies is to utilize epidemiological monitoring data as performance measures.<sup>9</sup> This can be accomplished by comparing published rates with in-house statistics for such measurements as the median MoM for each analyte, population parameters (log means and log standard deviations) and the initial and revised positive rates (see DS3.5.9.4).

**DS3.5.11 Failure rates for different sample types.** Few published data exist from screening programs, but kit manufacturers do provide information about acceptable sample types (e.g., serum vs. plasma), minimum sample volumes required, and conditions that can affect assay performance (e.g., hemolysis). Because laboratories should have specific sample processing protocols, many identifiable problem samples will be rejected before testing. Other testing “failures,” such as results falling below the lower limit of sensitivity of the assay due to a sampling error, are likely to be uncommon and resolvable by repeat testing. In rare cases, a second sample may be requested.

**DS3.5.12 External proficiency testing.** Each laboratory must participate in one or more of the external proficiency testing programs that evaluate assay performance for the serum analytes in the second trimester.<sup>24</sup> The proficiency testing should also include Down syndrome risk estimates (see Section C4).

**DS3.6 ANALYTIC VALIDITY.** The analytic validity of a test defines its ability to accurately and reliably measure a specific analyte that is to be used clinically. Each laboratory is responsible for documenting in-house validation of a test methodology using standard clinical chemistry criteria, which should include determination of inter- and intra-assay precision, establishment of linear range and the lower limit of detection, analytic specificity, and accuracy (e.g., recovery or method comparison). Information in the package insert of an FDA-approved kit or from the literature can be used as supporting evidence.

**DS3.6.1 Confirmatory testing.** Samples with results less than the lower limit of sensitivity of the assay must be repeated to rule out a technical error (e.g., sampling probe error) and to confirm the value. Results above the highest standard on the calibration curve must be repeated at dilution. Samples with a high coefficient of variation between replicate values (generally  $> 10\%$ ) are routinely retested by most laboratories to confirm the value. Confirmatory testing is a consideration for the majority of laboratories utilizing methodologies that test in duplicate, in order to minimize analytic errors.

**DS3.6.2 Assay robustness.** Assay robustness measures how resistant testing is to small changes in preanalytic and analytic variables. In an attempt to define performance requirements and minimize possible impact on assay performance (e.g., an-

alytic validity, reproducibility, failure rates), laboratories should consider the effects of common variables such as sample type, sample handling (e.g., transit time or conditions), sample quality, reagent lots, or minor changes in assay conditions (e.g., timing or temperature).

**DS3.7 CLINICAL VALIDITY.** The clinical validity of a genetic test defines its ability to accurately and reliably identify the clinical phenotype of interest. In this instance, it is the ability of the assigned Down syndrome risk (based on maternal age and multiple maternal serum analyte measurements) to identify pregnancies in which the fetus is affected with Down syndrome.

**DS3.7.1 Clinical sensitivity.** Clinical sensitivity (or detection rate) is the proportion of pregnancies with Down syndrome that has a positive test result (risk at or above the specified risk cut-off level).

**DS3.7.2 Clinical specificity.** Clinical specificity (or 1 – false-positive rate) is the proportion of unaffected pregnancies that has a negative test result (risk below the specified risk cut-off level).

**DS3.7.3 Screening performance.** Clinical sensitivity (detection rate) and the corresponding clinical specificity (1 – false-positive rate) will vary, depending on the combination of serum analytes used, the Down syndrome risk cut-off level chosen, the method of dating, and the distribution of maternal ages in the population being tested. Table 1 contains one published set of performance estimates for selected Down syndrome screening protocols that could be used for initial comparisons.<sup>2</sup>

**DS3.7.4 Positive and negative predictive values.** Positive and negative predictive values of testing in the target population measure the ability of the test to give accurate clinical information.

**Table 1**

Expected Down syndrome detection rate and false positive rate for two combinations of serum markers at three cut-off levels when dating is by last menstrual period or ultrasound measurements<sup>2</sup>

Screening cut-off and dating method	Maternal Age in Combination with	
	AFP, uE3, hCG(a)	a + DIA
<u>Last Menstrual Period Dating</u>		
1:350 term (1:270 2 <sup>nd</sup> )	70/6.6 <sup>a</sup>	75/5.0 <sup>a</sup>
1:300 term (1:230 2 <sup>nd</sup> )	68/5.6	73/4.3
1:250 term (1:190 2 <sup>nd</sup> )	65/4.6	71/3.5
<u>Ultrasound Dating</u>		
1:350 term (1:270 2 <sup>nd</sup> )	74/6.5	78/5.1
1:300 term (1:230 2 <sup>nd</sup> )	72/5.6	76/4.4
1:250 term (1:190 2 <sup>nd</sup> )	70/4.7	74/3.7

<sup>a</sup>Down syndrome detection rate/false positive rate.

**DS3.7.4.1 Positive predictive value.** The positive predictive value (PPV) is the proportion of positive test results that correctly identifies pregnancies with Down syndrome: [number of true positives / (true positives + false positives)]. The PPV can also be expressed as an odds ratio and is referred to as the odds of being affected given a positive result (OAPR).

**DS3.7.4.2 Negative predictive value.** The negative predictive value (NPV) is the proportion of negative tests that correctly identifies unaffected pregnancies: [number of true negatives / (true negatives + false negatives)]. Because the prevalence of Down syndrome is low, the NPV is generally not computed.

**DS3.7.5 Modifying factors.** Modifying factors are important to understand. These may be genetic, environmental, or other factors. Several of these have been discussed earlier and include maternal race (see DS3.5.4.2), family history (see DS3.5.7.4), and twin pregnancies (see DS3.5.7.2).

### DS3.8 RESULT REPORTING.

**DS3.8.1 Recommended report formats.** Final reports of test results (see Validation C8.5.7) must be clear to a nongeneticist professional and must include the following:

**DS3.8.1 (a)** Patient's name, date of birth and other unique identifiers;

**DS3.8.1 (b)** Name of referring physician/health center to receive the report;

**DS3.8.1 (c)** The test that is ordered;

**DS3.8.1 (d)** Type of specimen;

**DS3.8.1 (e)** Date when sample was obtained;

**DS3.8.1 (f)** Laboratory accession number(s) that uniquely identifies the sample;

**DS3.8.1 (g)** Demographic and pregnancy-related information used in the interpretation (e.g., gestational age, method of dating, maternal race, maternal weight);

**DS3.8.1 (h)** Analytic results in both mass units (e.g., ng/mL) and interpretive units (i.e., MoM) upon which all adjustments/corrections have been performed;

**DS3.8.1 (i)** Clinical interpretation, including whether the result is screen positive or screen negative, each analyte's MoM level, the risk cut-off level, and the patient-specific risk for Down syndrome.

**DS3.8.2 Reporting screen negative results.** Written reports of screen negative results can be transmitted to the referring physician by United States mail, courier, electronic transmission, or overnight carrier.

**DS3.8.3 Reporting screen positive results.** Screen positive results should be promptly transmitted to the referring physician, usually by phone and/or fax, within one working day after completion of the test. Appropriate recommendations for follow-up of screen positive results may include the following:

**DS3.8.3 (a)** Dating ultrasound (if not already done) to confirm gestational age and fetal viability;

**DS3.8.3 (b)** Genetic counseling;

**DS3.8.3 (c)** Referral for targeted ultrasound examination;

**DS3.8.3 (d)** Amniocentesis and karyotype;

**DS3.8.3 (e)** Given that amniocentesis has been performed, most laboratories will also measure amniotic fluid AFP and, if appropriate, acetylcholinesterase (AChE) testing for open neural tube defects (see ONTD).

**DS3.8.4 Reclassification of positive results.** Laboratories should be aware of the potential problems associated with reclassifying screen positive women as screen negative. There is a chance of reclassifying a true positive (the fetus has Down syndrome) as a false negative. Reclassification usually occurs when an LMP-dated pregnancy is subsequently dated by ultrasound and the difference between the LMP and ultrasound dating exceeds a set standard. As guidance to laboratories, reclassification should not be considered unless the revised estimate of gestational age is different by at least a week. Many laboratories use 10 days (e.g., 1.5 weeks) as the standard. One way to help avoid reclassification and improve overall screening performance is to encourage physicians to base their initial gestational age estimates on ultrasound measurements.

**DS3.8.5 Other conditions associated with a high Down syndrome risk estimate.**

**DS3.8.5 (a)** Delivery of an unaffected infant is the most common outcome associated with high Down syndrome risk;

**DS3.8.5 (b)** Overestimated gestational age (when the pregnancy is dated by last menstrual period);

**DS3.8.5 (c)** Uncommon chromosome abnormality (e.g., triploidy);

**DS3.8.5 (d)** Other fetal/placental/maternal abnormalities or conditions (these may occasionally be identified, but the strengths of these associations are low or may be due to coincidence.)

**DS3.8.6 Other conditions that can be identified in conjunction with a Down syndrome screening program.**

**DS3.8.6.1 Trisomy 18 (Edward syndrome).** Trisomy 18 is a serious chromosome abnormality that is nearly always fatal in the first few days or months after birth. In the absence of pre-

natal diagnosis and termination, the age-specific birth prevalence of trisomy 18 is about 10 times lower than for Down syndrome. An estimated 70% of trisomy 18 fetuses alive in the second trimester will be spontaneously lost by term. Maternal complications during delivery include a high rate of caesarian section. Although it would be difficult to justify a screening program aimed solely at identifying trisomy 18, such opportunistic testing is a common addition to second trimester serum maternal serum screening programs for Down syndrome. Because the pattern of markers is different in trisomy 18 compared to Down syndrome, a separate algorithm is required. Published algorithms are available that estimate an individual pregnancy's risk of trisomy 18.<sup>25</sup> Usually the false-positive rate is kept at 0.3% or lower, and the corresponding detection rate using maternal age in combination with AFP, uE3, and hCG measurements is about 70%. Maternal weight correction is an important component of trisomy 18 screening. Unlike Down syndrome screening, the marker pattern associated with trisomy 18 cannot be caused by incorrect gestational dating; therefore, redating the pregnancy is usually not informative.

**DS3.8.6.2 Steroid sulfatase deficiency.** In a small proportion of pregnancies, uE3 measurements will be below the lower limit of assay sensitivity (< 0.1 ng/mL or about 0.1 MoM). Although the majority of these observations will be explained (e.g., preexisting fetal death), about 1 in 1000 pregnancies will have no obvious reason for the low uE3 levels. Most of these remaining pregnancies will be males with steroid sulfatase deficiency. This deficiency manifests as X-linked ichthyosis (a mild to moderately severe skin disorder). Historically, these pregnancies had prolonged labor and perinatal complications, but these risks are minimal with current obstetrical care. There is no consensus as to whether this disorder meets the criteria for prenatal screening and intervention. It is reasonable for a laboratory to choose not to routinely comment on these results. Other rare metabolic disorders cause low uE3 measurements. The efficacy of testing for some of these disorders is currently being investigated.

**DS3.8.7 Subsequent ultrasound in the second trimester.** Before amniocentesis, some programs utilize ultrasound "soft markers" (e.g., shortened long bone measurements) to modify the Down syndrome risk assigned using the biochemistry results and maternal age. While this practice can reduce the number of women referred for amniocentesis, it will also reduce the program's detection rate. Screening programs should be aware of the advantages and disadvantages of this practice.

**DS3.9 CLINICAL UTILITY.** Clinical utility addresses the risks and benefits associated with testing in routine clinical practice. This information may be requested by those ordering or paying for testing, and the laboratory should be able to provide a reasonably accurate summary of the published literature. When clear gaps in knowledge exist, the laboratory may want to collect data in such a way as to address these questions. The

following is a list of selected clinical utility topics that often are applicable:

**DS3.9 (a)** Knowing whether pilot trials have been undertaken and, if so, what the results were;

**DS3.9 (b)** Establishing or adopting quality assurance processes that monitor the effectiveness of the laboratory's ongoing testing activities;

**DS3.9 (c)** Understanding possible adverse health or psychosocial consequences of testing;

**DS3.9 (d)** Describing what follow-up testing or interventions might be reasonable in persons with positive test results;

**DS3.9 (e)** Understanding what is known about the financial costs and economic benefits of testing.

**DS3.10 ETHICAL, LEGAL, AND SOCIAL IMPLICATIONS.** The laboratory should be familiar with the ethical, legal, and social issues regarding genetic testing in general, and those specifically applicable to maternal serum screening for Down syndrome. These may include informed consent, insurability, discrimination, labeling, confidentiality, obligations to disclose, and complex counseling issues. Legal issues such as patents, licensing, sample ownership and storage, proprietary testing, and reporting requirements should be carefully examined.

**DS3.11 ALTERNATIVES TO SECOND TRIMESTER MATERNAL SERUM SCREENING.** Several alternatives or additions to second trimester maternal serum screening have been proposed and are undergoing clinical validation. These include first trimester screening utilizing ultrasound measurements of nuchal translucency in combination with selected maternal serum markers.<sup>26,27</sup> This "combined" testing has the advantage of earlier diagnostic testing and performance that is comparable to second trimester quadruple marker testing. The best overall performance can be obtained by "integrating" the first trimester test results with the second trimester "quadruple" test and providing only a single interpretation in the second trimester (the integrated test).<sup>28</sup> Although not currently widespread in the United States, these alternatives are likely to become more widely adopted, and standards and guidelines will need to be developed to deal with these.

## DS4 EXISTING GUIDELINES

The American College of Medical Genetics issued policy statements entitled "Statement on Multiple Marker Screen in Women 35 and Older" (ACMG Newsletter, vol. 2, 1994) and "Statement on Multiple Marker Screening in Pregnant Women" (ACMG Newsletter 6:14, 1996). These documents are available online at <http://www.acmg.net>. Present Standards and Guidelines are in general agreement with those policy statements. Standards/guidelines from other organizations in-

clude the following: ACOG Technical Bulletin 228, September 1996; New York State Department of Health, Wadsworth Center, Laboratory Standards, Rev 12/2002; College of American Pathologists (CAP) Laboratory Accreditation Program, Special Chemistry Checklist, 2001 ([http://www.cap.org/apps/docs/laboratory\\_accreditation/checklists/checklistftp.html](http://www.cap.org/apps/docs/laboratory_accreditation/checklists/checklistftp.html)); New England Regional Genetics Group, Position Statement: Maternal Serum Screening for Down syndrome, Rev 6/1998. International Down syndrome Screening Group, Position Statement, 4/2001, (<http://www.leeds.ac.uk/idssg>).

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