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ACMG TECHNICAL STANDARD

Prenatal screening for trisomy 21 (Down syndrome) using first- and second-trimester biochemistry and nuchal translucency: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

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ABSTRACT

This technical standard was developed as a guide for laboratories performing prenatal screening for Down syndrome. It addresses 3 topics: second trimester (triple or quad), first trimester, including incorporation of nuchal translucency, and current directions in cell-free DNA screening. Analytic methods, clinical considerations, screening performance, guidelines for reporting second trimester, first trimester, integrated, contingent, and reflex screening tests for Down syndrome, are discussed. Individual laboratories are responsible for meeting the quality assurance standards described by the Clinical Laboratory Improvement Amendments, the College of American Pathologists, and other regulatory agencies, with respect to appropriate sample documentation, assay validation, general proficiency, and quality control measures.

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Introduction

Prenatal screening for Down syndrome (DS) using maternal serum has been approached in numerous ways over the past 40 years, and previous technical standards have addressed different phases and types of maternal serum screening in separate documents. This document addresses second-trimester[1-4] and first-trimester stand-alone[5-8] screening, as well as protocols that combine markers from both the first and second trimesters, such as integrated screening,[9] serum integrated screening,[10] sequential screening,[11] and contingent screening.[12] It is intended to represent a comprehensive summary of current serum screening practices within the United States and globally at a time when most research toward serum marker development has reached a steady state. In addition, we include protocols that anticipate serum screening to be followed by prenatal cell-free DNA screening.[13,14]

Background

Clinical description and etiology

DS (OMIM 190685) is the most common genomic disorder associated with intellectual disability and results from an extra copy of chromosome 21, most commonly from the free chromosome and less frequently from a translocation or other structural chromosome abnormality. Manifestations of DS affect many body systems and include neurodevelopmental, musculoskeletal, skeletal, cardiovascular, and other features, including intellectual disability, developmental delays, hypotonia, characteristic facial features, and congenital heart defects, with an increased risk for adverse health conditions, including hypothyroidism, Alzheimer disease, leukemia, recurrent infections, and decreased longevity.

Incidence and inheritance

DS is identified in approximately 1 in 500 live births, with 95% of cases resulting from sporadic nondisjunction during parental meiosis (most often maternal [95%]) and occurring during meiosis I (75%). The risk of having a child with DS increases with advancing maternal age. Table 1[15-17] contains recent estimates of the prevalence of DS and other common autosomal aneuploidies at term, as well as the number of expected cases in the first and second trimesters. This table uses the maternal ages at birth in the United States in 2018.

Prenatal screening for Down Syndrome

Various screening tests for DS have been available in the first and/or second trimester. Screen-positive pregnancies are then referred for an invasive procedure such as chorionic villus sampling (CVS) or amniocentesis followed by diagnostic testing via fetal karyotyping or molecular testing (eg, fluorescence in situ hybridization or quantitative fluorescent polymerase chain reaction). Down syndrome screening combines biochemical measurements in maternal serum with other clinical and biometric data to generate a patient-specific risk for fetal DS. This was first implemented in second-trimester pregnancies in the mid-1980s using levels of second-trimester maternal serum alpha-fetoprotein (AFP) measurements. Soon after, additional second-trimester markers, such as unconjugated estriol (uE3), human chorionic gonadotropin (hCG), and dimeric inhibin-A (DIA)[18-24] were included, along with adjustments for maternal age and other factors, including maternal weight and race. Research into biomarkers and modalities for DS screening was a dynamic area of study for 3 or more decades beginning in the mid-1980s. These studies resulted in the identification of dozens of potential second-trimester markers from blood and saliva; however, serum screening using a combination of AFP, uE3, hCG, and DIA in the second trimester was well established as the most common second-trimester screen test for DS.

This second-trimester approach was expanded to the first trimester by incorporating measurements of fetal nuchal translucency (NT) (the subcutaneous collection of fluid in the fetal nuchal region), with first trimester maternal serum markers, such as pregnancy-associated plasma protein A (PAPP-A), and either total or intact hCG or its beta subunit (free beta-hCG).[5-7] Less frequently, DIA,[25,26] hyperglycosylated hCG,[27,28] and placental growth factor[29] have also been included in the first trimester screen. The association of NT with an abnormal fetal karyotype was reported in 1989 by Bronshtein et al[30] and in 1990 by Szabó and Gellén.[31] By 1991, it had been demonstrated that NT measurement could serve as a useful screening marker for fetal DS.[32-34]

Prenatal DS testing performance improved dramatically with advancements in detecting placental-derived cell-free DNA (cfDNA) in maternal serum. In 1997, cfDNA from placental cells was detected in maternal serum as circulating Y-chromosome sequences in pregnancies with male fetuses but not female fetuses.[35] In 2008, 2 groups provided proof of concept for DS screening using cfDNA.[36,37] In 2011, prenatal cfDNA screening was offered clinically, based on an external validation study of 211 confirmed cases of DS and 1484 matched euploid singleton pregnancies.[38] Subsequent validation studies in singleton pregnancies demonstrated reliable detection of trisomy 21, as well as trisomies 18 and 13, with detection rates (DR) of 99.2%, 96.3%, and 91.7% and a combined false-positive rate (FPR) of 0.32%.[39-41] However, a few percent of samples resulted in a test failure.[42] More recently, clinical cfDNA tests have advanced to identify fetal sex, as well as sex chromosome aneuploidies, rare autosomal trisomies, and selected or genome-wide microdeletions or duplications. The American College of Medical Genetics and Genomics (ACMG) has published evidence-based guidelines for the use of prenatal cfDNA screening[43] as a primary screening test and a Position Statement on its use in clinical care.[44]

In late 2022, cfDNA was the primary screen for 2.18 million pregnancies in the United States (~60% of all

Table 1 Age-associated risks and expected number of common trisomies, based on published rates applied to 2018 United States liveborn birth data

Age	US 2018	Term DS	Number of DS			Term T18	Number of T18 at			Term T13	Number of T13 at		
Comp years	Liveborn	Risk (1:N)	First	Second	Term	Risk (1:N)	First	Second	Term	Risk (1:N)	First	Second	Term
15	6689	1513	7.8	5.7	4.4	9029	2.6	2.1	0.7	13,741	1.0	0.8	0.5
16	13,088	1509	15.2	11.3	8.7	9022	5.2	4.1	1.5	13,731	1.9	1.6	1.0
17	26,250	1504	30.6	22.7	17.5	9013	10.4	8.3	2.9	13,717	3.8	3.3	1.9
18	50,088	1497	58.7	43.4	33.4	9001	19.9	15.9	5.6	13,698	7.2	6.3	3.7
19	85,492	1488	100.8	74.6	57.4	8984	34.0	27.2	9.5	13,671	12.3	10.8	6.3
20	112,264	1477	133.4	98.7	76.0	8961	44.7	35.8	12.5	13,634	16.1	14.2	8.2
21	128,179	1461	153.9	113.9	87.7	8929	51.3	41.0	14.4	13,582	18.5	16.3	9.4
22	146,171	1441	177.9	131.7	101.4	8886	58.8	47.0	16.5	13,510	21.2	18.7	10.8
23	162,207	1415	201.0	148.8	114.6	8826	65.6	52.5	18.4	13,411	23.7	20.9	12.1
24	177,354	1382	225.1	166.6	128.3	8744	72.4	58.0	20.3	13,275	26.2	23.0	13.4
25	193,717	1340	253.7	187.8	144.6	8632	80.1	64.1	22.4	13,089	29.0	25.5	14.8
26	208,541	1287	284.4	210.5	162.1	8481	87.8	70.3	24.6	12,838	31.9	28.0	16.2
27	223,066	1221	320.6	237.3	182.7	8279	96.2	77.0	26.9	12,499	35.0	30.8	17.8
28	235,469	1141	362.1	268.0	206.4	8011	105.0	84.0	29.4	12,052	38.3	33.7	19.5
29	238,698	1047	400.0	296.1	228.0	7662	111.3	89.0	31.2	11,470	40.8	35.9	20.8
30	238,759	939	446.0	330.2	254.2	7214	118.2	94.6	33.1	10,734	43.6	38.4	22.2
31	235,363	821	503.1	372.4	286.8	6657	126.3	101.0	35.4	9831	46.9	41.3	23.9
32	223,605	696	563.3	417.0	321.1	5988	133.4	106.7	37.3	8771	50.0	44.0	25.5
33	207,447	572	635.8	470.6	362.4	5219	142.0	113.6	39.8	7587	53.6	47.1	27.3
34	185,523	456	714.0	528.5	407.0	4382	151.2	121.0	42.3	6346	57.3	50.4	29.2
35	165,513	353	823.3	609.5	469.3	3530	167.5	134.0	46.9	5132	63.2	55.6	32.3
36	138,958	267	914.2	676.7	521.1	2725	182.1	145.7	51.0	4030	67.6	59.5	34.5
37	110,624	199	975.8	722.3	556.2	2023	195.3	156.3	54.7	3101	69.9	61.5	35.7
38	86,166	148	1021.8	756.4	582.4	1457	211.2	169.0	59.1	2369	71.3	62.7	36.4
39	65,525	111	1035.5	766.5	590.2	1033	226.5	181.2	63.4	1824	70.5	62.0	35.9
40	46,286	85	956.4	708.0	545.1	734	225.2	180.2	63.1	1432	63.4	55.7	32.3
41	32,084	67	844.1	624.8	481.1	531	215.6	172.5	60.4	1158	54.3	47.8	27.7
42	20,022	54	650.3	481.4	370.7	397	180.0	144.0	50.4	969	40.5	35.6	20.7
43	12,169	45	472.6	349.8	269.4	309	140.5	112.4	39.3	838	28.5	25.0	14.5
44	6820	39	307.1	227.3	175.0	251	96.9	77.5	27.1	747	17.9	15.7	9.1
45	3896	35	197.8	146.4	112.8	213	65.4	52.3	18.3	685	11.2	9.8	5.7
46	2158	31	120.6	89.3	68.8	187	41.3	33.0	11.6	641	6.6	5.8	3.4
47	1331	29	80.2	59.4	45.7	169	28.2	22.5	7.9	610	4.3	3.8	2.2
48	752	27	48.1	35.6	27.4	156	17.2	13.7	4.8	588	2.5	2.2	1.3
49	479	26	32.1	23.7	18.3	148	11.6	9.3	3.2	572	1.6	1.4	0.8
50	959	25	66.5	49.2	37.9	142	24.2	19.3	6.8	561	3.3	2.9	1.7
Total	3,978,497		14,134	10,463	8,056		3,545	2,836	993		1,135	998	579
Computed	1 in		281	380	494		1,122	1,403	4,008		3,505	3,987	6,873
	per 100,000		355	263	202		89	71	25		29	25	15

Births to mothers under age 15 and those above age 50 are included in the first and last categories, respectively. US birth data can be found at https://wonder.cdc.gov/natality-current.html. The Down syndrome age-associated risks are found in Snijders.[15] The T18 and T13 age-associated risks are found in reference Morris and Savva.[16] Spontaneous loss rates for all 3 common trisomies can be found in reference Hecht and Cook.[17]

Table 1 can be used in the following ways:

- Given a woman's truncated estimated age at delivery of 32, read across that row to find the number of live births occurring in 2018 for that age (233,605).
- The first box shows:
  - The term Down syndrome risk for a woman of that age (1 in 696).
  - The estimated number of first trimester (12 weeks) cases in the United States in 2018 (563).
  - The estimated number of second-trimester cases (18 weeks) (417).
  - The estimated number of term cases (321).
  - At the bottom of the 3 columns of estimated numbers are the following:
    - Total number of cases at the 3 times in pregnancy (14,134, 10,463, and 8,056).
    - Overall risk for the population is provided at the 3 time periods of pregnancy as both an odds (1 in 281) and as a rate per 100,000 (355).
- The second box shows the same data for trisomy 18.
- The third box shows the same data for trisomy 13.

deliveries), whereas serum-based screening was utilized by about 0.50 million pregnancies (~14%).[45] However, outside of the United States, several laboratories reported routinely using serum screen as a primary test having a high screen-positive rate with a follow-up cfDNA test. Thus, it is important to maintain high quality serum/ultrasound-based screening, at least for the near future.

The approaches of screening and diagnostic testing are distinct, with different expectations for clinical sensitivity, clinical specificity, cost, and acceptable level of risk. Screening tests using maternal serum markers with or without NT measurements are intended to identify pregnancies at sufficiently high risk to warrant further diagnostic testing, which involves invasive sample collection via amniocentesis or CVS. The DR (or clinical sensitivity) and FPR (or 1-clinical specificity) depend on maternal age, analytes measured, distribution of markers in affected and unaffected populations, assay precision, methods for measuring NT, and determining gestational age, appropriateness of reference data, and cut-off levels for screen-positive results. Long-term monitoring of both laboratory and ultrasound measures should be part of internal and external laboratory quality assurance. Test performance for fetal DS detection using cfDNA has sufficiently high DR and low FPR that some may consider diagnostic, but it is not. Both false-positive and false-negative results occur.[41]

Prenatal DS screening comprises a comprehensive program encompassing preanalytic, analytic, and postanalytic components. Section 1 of this guideline describes methods for DS risk assessment that combines maternal age-associated risk with biochemical testing of DS markers in maternal serum. Section 2 describes methods for incorporating NT into first-trimester screening, including the collaboration between laboratories and sonographers, and considerations for integrated and sequential approaches to screening. Section 3 briefly describes methods and performance for incorporating prenatal cfDNA screening as a follow-up to an abnormal serum/ultrasound screening result for DS. Established general principles for sound laboratory practice are found in the ACMG Technical Standards for Clinical Genetics Laboratories.[46] Laboratories should be aware that early second-trimester serum screening for open neural tube defects is likely to continue to be a relevant offering during pregnancy.[47] A list of other current prenatal serum screening guidelines may be found in Supplement 1.

Materials and Methods

This laboratory technical standard was informed by a review of the literature, including current guidelines, and expert opinion. Resources consulted included PubMed (search terms: prenatal screening, maternal serum screening, aneuploidy screening, laboratory guideline, cell-free DNA, non-invasive prenatal screening, and noninvasive prenatal testing), the ACMG Standards and Guidelines for Clinical Genetics Laboratories, Clinical and Laboratory Standards Institute guidelines, and Clinical Laboratory Improvement Amendments (CLIA) regulations. When the literature provided conflicting evidence about a topic or when there was insufficient evidence, the authors used expert opinion to inform the recommendations. Expert opinion included the coauthors of the document and members of the ACMG Laboratory Quality Assurance Committee.

Any conflicts of interest for workgroup members or consultants are listed. A draft was presented to the ACMG Board of Directors for review and subsequently for member comment. The draft document was posted on the ACMG website, and an email link was sent inviting ACMG members to provide comments. The authors assessed all comments. When appropriate, additional evidence was included to address member comments, and the draft was amended. Both member comments and author responses were reviewed by a representative of the ACMG Laboratory Quality Assurance Committee and by the ACMG Board of Directors. The ACMG Board of Directors approved the final document before submission for publication.

Section 1: Maternal serum testing in the first and/or second trimester

Preanalytical requirements

Pretesting laboratory responsibilities

Educational materials for patients and providers. Laboratories must make educational materials on DS screening available to patients and their providers. Many laboratories and professional organizations (eg, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors) and regional genetics groups have developed educational materials with general information about the target conditions, screening performance, patient rights, eligibility, test interpretation, treatment options, costs, risks and benefits of testing, and steps to anticipate following a positive result. Such material should be at no higher than the 8th grade reading level and be translated into those languages commonly used by screened patients.

Informational materials for health care providers. Laboratories must provide pretesting information to clients, including instructions for sample collection, labeling and transportation, test requisitions, and information about turnaround time, population-based expectations for clinical test performance (DR, FPR, and turnaround time), and reporting formats.

Informed consent. Laboratories must provide sufficient information about prenatal screening to the health care provider for them to obtain proper informed consent. It is the role of the ordering health care professional, not the laboratory, to inform patients about the benefits and limitations of prenatal screening and to obtain consent. In some instances (eg, New York State), the laboratory may be required to document attempts at obtaining consent.

Requisition forms and reports. Sufficient clinical information must be obtained to ensure accurate results interpretation, including basic demographic information as specified in the ACMG Technical Standards for Clinical Genetics Laboratories,[46] as well as information specific to

prenatal DS screening, such as gestational age, maternal weight, race, parity, family history, ultrasound measures, smoking status, etc. Reports must contain all standard elements and should be clear to a nongeneticist. A detailed list of essential information for prenatal screening and basic reporting elements is detailed in Supplement 2.

Specimen collection, transportation, and storage

Specimen types. Serum is the most common sample type for most assays. Dried blood spots (DBS) require less invasive collection methods and are easier to transport than serum and may improve screening performance for free beta-hCG because of stability issues.[48] However, the performance of PAPP-A and hCG is lower in DBS than serum.[49] The laboratory must validate all sample types in use for clinical testing.

Sample handling and storage. The laboratory must specify procedures for acceptable specimen handling from the point of collection to the laboratory, including packaging, shipping conditions, and mode of transportation. Serum and DBS markers are relatively stable,[49-51] and both sample types can be shipped at ambient temperature. AFP, uE3, DIA, PAPP-A, and the intact (or total) forms of hCG can be reliably determined in serum stored at 4 °C to 8 °C for at least 6 days[52] and at −20 °C for several months. For optimal performance, shipping time should be minimized and exposure to high temperatures avoided. When measuring free beta-hCG in serum, samples must be protected from high temperatures (eg, cool packs with overnight shipment in the summertime). The use of DBS can also result in improved stability of free beta-hCG.[49] See Supplement 3 for additional information.

Criteria for sample rejection. Criteria for sample rejection must be established by the laboratory and relate to clinical considerations (eg, gestational age out of range) or characteristics of the sample itself (eg, inappropriate sample type, delayed transit time, insufficient quantity, and gross hemolysis).

Test validation

Test and screening performance

Analytic validation

Laboratories are responsible for documenting in-house validation of analytic performance in accordance with CLIA, including inter- and intraassay precision, linearity, lower limit of detection, analytic specificity, and accuracy. Effects of common variables such as sample type, sample handling (eg, transit time or conditions), sample quality, reagent lots, or minor changes in assay conditions (eg, timing or temperature) should also be evaluated. Information in the package insert of a US Food and Drug Administration (FDA)-approved kit or from the literature may be used as supporting evidence.

Screening performance

Clinical sensitivity, or DR, is the proportion of affected pregnancies that test positive (individual risk at or above the specified risk cut-off level), whereas clinical specificity is the proportion of unaffected pregnancies that test negative (risk below the specified cut-off). The DR and FPR depend on various factors, including the combination of serum analytes used, screening cut-off, and distribution of maternal ages in the tested population. Published studies compare various marker combinations and risk cut-offs. Table 2[53] contains performance estimates for selected first and second-trimester DS screening protocols, based on published parameters,[25,53-58] and should be used only as a guide for initial comparisons.

The positive predictive value (PPV) and negative predictive value (NPV) measure the test's ability to give accurate clinical information for a given population. The PPV is the percentage of positive test results that correctly identify a DS (true positives/(true positives + false positives)) × 100. The PPV can also be expressed as an odds, also called the odds of being affected given a positive result (OAPR).

The NPV is the percentage of negative tests that correctly identify an unaffected pregnancy (true negatives/(true negatives + false negatives)) × 100. Because the prevalence of DS in a screening population is low (eg, 1:500 at term in the general US population), the NPV is generally not computed or reported.

Additional material related to screening performance is provided in Supplement 4.

Table 2 Expected screening performance for 1st trimester, 2nd trimester screening markers, and the combination of the best markers in each trimester

Test	Trimester(s)	FPR (%)	DR (%)	Risk (1:N)[a]	PPV[b]
Combined[c]	1st	**5.0**	83	1:270	1:27
Quadruple[d]	2nd	**5.0**	81	1:210	1:27
Integrated (serum)[e]	1st and 2nd	**5.0**	86	1:250	1:26
Integrated[f]	1st and 2nd	**5.0**	93	1:400	1:24
Combined	1st	3.7	**80**	1:180	1:21
Quadruple	2nd	4.5	**80**	1:180	1:25
Integrated (serum)	1st and 2nd	2.9	**80**	1:80	1:16
Integrated	1st and 2nd	0.6	**80**	1:45	1:3
Combined	1st	3.8	81	**1:200**	1:21
Quadruple	2nd	4.7	82	**1:200**	1:30
Integrated (serum)	1st and 2nd	4.1	83	**1:200**	1:22
Integrated	1st and 2nd	2.4	89	**1:200**	1:12

Data extracted from published modeling.[53] Boxed entries are held constant over the range of tests. If intact hCG were substituted for fβhCG in the combined test, estimates would be slightly lower.

[a]Risk as of the early 2nd trimester (16-18 weeks).

[b]Positive predictive values expressed as an odds (eg, 1:27, risk = 1/[N+1] or 1/28 or 3.6%).

[c]Combination of maternal age, NT, PAPP-A, and fβhCG at 12 weeks.

[d]Combination of maternal age, AFP, uE3, hCG, and DIA.

[e]Combination of the quadruple test with 1st trimester PAPP-A.

[f]Combination of the serum integrated test with 1st trimester NT.

Kits and reagents

The FDA has not licensed any kits intended for second-trimester Down syndrome screening. Most kits have class 2 approvals (510K) for other clinical applications, which restrict manufacturers from making clinical claims about DS. The National Committee for Clinical Laboratory Standards has published procedures for evaluating manufactured kits, including precision, linearity, stated performance characteristics, and guidelines on DR and FPR.[59] Several AFP kits are commercially available (licensed by the FDA to screening for open neural tube defects) in immunometric format. All can reliably measure AFP in the range relevant to DS screening (10-50 IU/mL),[60] as well as open neural tube defects (approximately 100 IU/mL). Kits for measuring uE3 and hCG are also available, whereas fewer options exist for purchasing kits for measuring DIA and free beta-hCG. The kit used for DIA must target levels between 10 to 1000 pg/mL, the range relevant to DS screening.

For the first trimester, PAPP-A, free beta-hCG, and intact hCG are not licensed by the FDA for specific use in DS screening. Moreover, the FDA has not licensed PAPP-A or free beta-hCG reagents for any use, and testing must be performed using research use only kits, laboratory developed tests, or analyte-specific reagents. Only hCG (intact and total) kits have class 2 approvals (510K), albeit for applications other than DS screening. In the United States, manufacturers are thus restricted from making clinical claims about DS screening. Research use only kits are available for serum PAPP-A and intact or total hCG assays. Because of patent and licensing agreements, options for purchasing analyte-specific reagents for measuring DIA, hyperglycosylated hCG, and free beta-hCG in serum, as well as PAPP-A, free beta-hCG, and intact hCG in DBS, are limited.

All reagents for maternal serum screening must meet performance requirements exceeding those for other intended uses, and results must have both high accuracy and precision. Different kits need not give identical values on the same sample, provided in-house reference data (ie, medians) are established using the same kit in an appropriate population. Coefficients of variation of more than 10% can adversely affect both detection and screen-positive rates. It is also important to ensure the stability of kits and reagents over a long period of time, and to minimize lot-to-lot variability. Specific details regarding standards and calibration are given in Supplement 5.

Quality control

The laboratory must develop a robust quality control program using materials either from kits or made in-house.

Assay controls

Commercially available controls, including those in kits, can serve as checks on reagents and technical performance. Long-term monitoring can be achieved with commercial controls bought in sufficient quantity to last a year or more. Commercial uE3 controls may require dilution with nonpregnant serum to achieve concentrations appropriate for second-trimester testing. No commercial controls are available for DIA measurements or for DBS assays. Liquid controls may be used for DBS assays to check for reagent and technical performance. In-house DBS controls should be made to check for extraction efficiency.

In-house pooled serum controls

In-house pooled controls provide a sample matrix resembling patient samples and have screening marker concentrations near the clinical action points for DS (ie, lower uE3 and PAPP-A, higher hCG, or free beta-hCG). Large control lots with long expiration dates can aid in long-term assessments of kit master reagent lot changes and assay drift.

Repeat assay controls

Repeat assay controls can be helpful in monitoring long-term performance variability. Short-term intra- and interassay precision may be assessed by retesting patient samples chosen at random from recent assay batches. Because there is minimal change in currently measured serum analytes when frozen and thawed, retesting stored patient samples from the time when median values were established can also reveal long-term drift and the need to update reference data. If frozen samples are used to derive medians, however, the laboratory should document the stability of measurements of each analyte over time.

Assay quality control

Each assay should contain at least 2 quality control samples that fall at clinical action points (3 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 (MoM) at 16 weeks, along with a second control near the median (1.0 MoM). In the first trimester, an hCG or free beta-hCG high control could be targeted at a 2.0 or 2.5 MoM value at 12 weeks, along with a second control near the median (1.0 MoM).

Performance targets for in-house pooled controls can be set using standard clinical laboratory quality control approaches.[61] Controls from licensed kits have an acceptable target range specified by the manufacturers, but laboratories may elect to establish an in-house range. This information is used to accept or reject individual control results or a whole assay, and ranges should be set with care to avoid unnecessary result rejection.

Proficiency testing

Participation in an ongoing proficiency testing program is required by CLIA and allows for continual monitoring and evaluation of testing quality. Laboratories must participate in a proficiency testing or interlaboratory comparison program that evaluates assay performance for the serum analytes in the first, second, or both the first and second trimester(s).[62,63] Proficiency testing should include computations for

maternal age, gestational age, and DS risk estimates. All proficiency samples must be incorporated into the regular clinical workflow and handled, analyzed, and reviewed in the same manner as all other clinical specimens in the laboratory.

Clinical utility

Clinical utility addresses the risks and benefits of testing in routine clinical practice. The laboratory should be able to provide a summary of supporting literature on test utility to ordering providers or payors and should consider addressing any knowledge gaps by collecting additional data. Additional notes related to clinical utility are detailed in Supplement 6.

Ethical, legal, and social implications

The laboratory should be familiar with the ethical, legal, and social issues related to genetic testing in general, and maternal DS serum screening specifically. These include informed consent, insurability, discrimination, labeling, confidentiality, and obligations to disclose. Legal issues, including patents, licensing, sample ownership and storage, proprietary testing, and reporting requirements, should be carefully examined. Educational materials must be free from commercial or marketing influences.[64]

Results interpretation and reporting

Gestational age

Obtaining an accurate gestational age is critically important to screening performance. Biochemical and ultrasound markers fluctuate during pregnancy, and extreme care must be taken both to determine the gestational age of the fetus as accurately as possible, and to establish and monitor the median values that serve as norms for each marker used in the risk estimate for DS.

Computing medians

Weighted regression of empirically determined medians using established models and determination of medians specific to each gestational day improves median accuracy. In the second trimester, AFP and uE3 fit a logarithmic linear model, intact hCG and the free beta subunit of hCG an exponential model, and DIA a log cubic or quadratic model. In the first trimester, all analytes fit a log-linear model between 11 and 13 weeks' gestation.[65]

Converting results to MoM

To interpret screening results, serum marker concentrations in mass units (ng/mL) or international units (IU/mL) must be converted to MoM levels based on gestational age. The laboratory must establish internal normative data (see Quality Assurance section below) or, at a minimum, demonstrate that data obtained from another source are appropriate for its screened population. This would ideally require testing 100 samples for each gestational week (weeks 11-13 for first trimester, and weeks 15-20 for second trimester). Package insert (ie, commercial) medians should not be used, even for a short time. MoM levels may be further adjusted for other factors as described below. Methods for reliable median determination are given in Supplement 7.

Impact of gestational age on MoM

For second-trimester screening, the DR and associated FPR at a select risk cut-off level are essentially constant between 15- and 20-weeks' gestation. The optimal screening window is set at 16 to 18 weeks to accommodate the concurrent use of AFP in screening for open neural tube defects. For samples collected at 14 weeks' gestation, the report should note the need for a later sample for open neural tube defect screening. Under exceptional circumstances, laboratories may accept samples later than 20 weeks' gestation with the understanding that clinical management options may be limited.

For first trimester screening, the DR and associated FPR at a select risk cut-off varies by gestational week. Screening performance declines slightly from weeks 11 through 13. Measurements of both NT and PAPP-A perform worse, whereas free beta-hCG and hCG perform better, as gestational age increases from 11 through 13 completed weeks' gestation.[25] Detailed methods for estimating gestational age are given in Supplement 8.

Methods of assessing gestational age

Crown rump length (CRL) provides an unbiased estimate of gestational age relative to DS and is the standard for estimating gestational age in the 1st trimester. However, there may be slight differences in the gestational age estimate from a given CRL depending on the software used. Biparietal diameter is preferred in the 2nd trimester because it improved the detection of open spina bifida.

Factors used to adjust MoM levels

Although less critical than gestational age in optimizing interpretation, interpretive refinements may be based on patient demographics.

Laboratories should utilize published weight adjustment formulas only until in-house data are collected and new laboratory-specific formulas are derived.[66]

Adjustment for maternal race/ethnicity should be made for AFP and hCG measurements either by establishing medians for each subpopulation, or by applying established mathematical corrections available within the published literature. Such corrections have been challenged in the literature for AFP measurements,[67] but a comprehensive analysis of a large recent data set confirms the need for correction.[68] It would be premature to consider the matter of race corrections in screening to be closed.

The impact of insulin-dependent diabetes mellitus (IDDM) on AFP levels was initially reported to be a 25% reduction in AFP levels,[69,70] and many laboratories corrected AFP for maternal IDDM by using an adjustment factor for AFP MoM levels; however, more recent data are conflicting, and there is no consensus on correction for gestational diabetes.[71-73]

Correction for smoking is recommended for both first and second-trimester markers. In the second trimester, correction is needed to avoid unnecessarily high screen-positive rates for DS. In the first trimester, correction is needed to avoid unnecessarily high screen-positive rates for trisomy 18 when using the combined or integrated tests.[74] Accounting for smoking status when interpreting risks is up to the discretion of the laboratory director and may depend on the specific software used for risk analysis. At this time, laboratories should consider adding a simple yes/no question concerning cigarette smoking status to their requisition slip. Methods to incorporate such information in a DS screening program and the expected results have been published and can be used as a guide.[74]

In the second trimester, there is a clear effect of pregnancies achieved by in vitro fertilization (IVF) on serum screening markers. Maternal serum levels of uE3 decreased by about 10%, whereas hCG and DIA increased by about 10% in IVF relative to spontaneous pregnancies. Without adjustment, this would lead to an inappropriately high DS screen-positive rate in IVF pregnancies.[75,76] With egg donation, the age of the donor is substituted for maternal age when calculating the a priori risk.[77] Impacts of these adjustment factors are detailed in Supplement 9.

Risk calculation

Patient-specific DS risks are generated by complex algorithms integral to prenatal screening. These software applications can be commercially obtained or developed in-house and must be verified before routine clinical use and after each software update. Common algorithms use adjusted MoM values to calculate a likelihood ratio based on the overlapping multivariate Gaussian distributions defined by the affected and unaffected population parameters. The a priori risk for DS, based on maternal age at term, is then multiplied by the patient's likelihood ratio to calculate a patient-specific risk. Equations to compute the a priori risk for a given maternal age have been published.[78,79] It is also possible to provide risks specific to the late first trimester, early second trimester, or at term using published rates of spontaneous loss.[15,16,78]

Risk algorithms for DS use published or in-house population parameters for each of the analytes, expressed as log means and log standard deviations for unaffected and affected pregnancies.[19,55,56,80] Pairwise correlation coefficients in both affected and unaffected pregnancies and truncation limits are needed to generate accurate and reliable risks. These parameters vary with gestational age in the first trimester but not in the second trimester, and the use of gestational age week-specific parameters in the first trimester is recommended. DS risks should be computed for maternal age in decimal years; published equations can be used.[17,78,81]

Risks can be expressed relative to DS prevalence in either the trimester at which the sample is taken (1st or 2nd), or at term. Calculation of the 1st trimester risk is achieved by multiplying the term risk by 1.75 and in the 2nd trimester, multiplying by 1.3. The lower risks at term compared with 1st and 2nd trimester are due to the likelihood of pregnancy loss. Additional details on calculating 1st and 2nd trimester risks are found in Supplement 10.

Other variables affecting risk assessment

Screening performance is influenced by the timing of sample collection, as well as other factors. The optimal time for screening in the second trimester is 16 to 18 completed weeks, and in the first trimester (based on serum testing alone), it is 11 completed weeks or even earlier if using free beta-hCG.[25,55] PAPP-A measurements are most discriminatory at 9 weeks' gestation. Beyond 18 weeks, risk assessment may still be possible but screening the options are limited when a positive result is found.

In both the first and second trimester, DS screening in twin pregnancies is less effective than for singleton pregnancies. Serum marker levels in twin pregnancies reflect the overall pregnancy, whereas first trimester NT measurements are specific to the fetus. A family history of DS increases the a priori risk, depending on the degree of relatedness and the mode of inheritance. Additional guidance for assigning screening risk for multiple gestation and considering family history is given in Supplement 10.

Risk cut-offs

Historically, diagnostic testing after CVS or amniocentesis was offered to patients aged 35 years or older at term (eg, term risk of 1:350; second-trimester risk of 1:270, first trimester risk of 1:200). Second-trimester screening programs combining AFP, uE3, hCG, and DIA with maternal age may continue using the second-trimester risk cut-off of 1:270. It is also acceptable to choose a higher (eg, 1:200) or lower (eg, 1:300) risk cut-off level.[55]

First trimester screening programs combining NT measurements with PAPP-A and free beta-hCG or hCG in combination with maternal age (at 11-13 completed weeks, combined test) may use the risks cut-off level equal to that of a 35-year-old patient in the first trimester (eg, 1:200). Similar to that in the second trimester, it may also be acceptable to choose a higher or lower risk cut-off level (eg, first-trimester risk of 1:150).[55]

Repeat testing

Obtaining a second specimen for repeat testing as part of DS screening in the first or second trimester is discouraged, except for cases of suspected sample mix-up. If an initial sample is later found to have been collected too early, the subsequent sample should be considered the first interpretable sample. Laboratories should not interpret a known repeat sample as if it were an initial specimen. Published methods exist to combine the information from the 2 samples to provide a more reliable interpretation.[82,83]

In contrast, individual samples with results less than the lower limit of assay sensitivity should be repeated to exclude technical error and to confirm results. Results above the highest calibration standard must be repeated at dilution. When tested in duplicate, samples with a high coefficient of

variation between replicate values (generally, >10%) are routinely retested by most laboratories to confirm the value. Most laboratories use automated platforms that test in singlicate.

Results reporting

Final reports of test results should be clear to a nongeneticist professional and must include specific information used in determining the risks. All of the following should be collected: gestational age and method of its determination, maternal weight, race/ethnicity, parity, family history of DS or neural tube defects, NT, CRL, sonographer and sonography center names, and smoking status. Information on IDDM and assisted reproductive technology may also be useful.

When critical information is not provided, the report should indicate that information was missing and which information was used in the interpretation. Sometimes it may be appropriate to include the potential impact of missing information on the report (eg, maternal weight), whereas in other cases, a full interpretation may not be possible (eg, missing maternal age or gestational age).

For screen-negative results, written reports can be transmitted to the referring physician by electronic transmission, secure online portals, or by mail/courier. For screen-positive results, results should be provided within 1 business day and should include recommended follow-up, including genetic counseling and consideration of diagnostic or other follow-up testing. For additional details, see Supplement 11.

Quality assurance

Long-term assessment of variability and performance

Normative data review

Laboratories should review median values at regular intervals, preferably monthly, if sample volumes allow and recalculate when necessary. Medians should be recalculated if analyte values shift more than 10% or demonstrate consistent change more than 5% over current medians whether due to observed assay drift or reagent lot change. Shifts in analyte values can be monitored through epidemiological monitoring by computing the overall median MoM level over a specified interval. Only if such monitoring shows that the median MoM has been stable should observations from samples tested in the previous months or years be used. Alternative methods of revising medians may be necessary if a significant shift has been observed.

Evaluating medians with new reagent lots

A set of 25 to 50 patient samples and control samples should be run on the old and new kit/reagent lots and results analyzed using regression analysis and formal method comparison.[84] That correlation can be applied to existing medians to derive temporary new medians, which can be used until sufficient data are available from the new lot to calculate new medians.

Epidemiological monitoring

To monitor assay and program performance for identifying areas of concern, screening programs must perform epidemiological monitoring by gathering data from the screened population to calculate metrics (eg, FPR) to use as extended quality control measures.[85] Minimally, this should include periodic computation (monthly or weekly depending on sample numbers) of the median MoM for each analyte and determination of the statistical significance of any deviation from 1.00. All corrective actions must be documented. Laboratories should periodically compute their initial positive rate and compare it with expected published rates after accounting for the specific combination of analytes and the risk cut-off level.

Long-term monitoring of test performance

Whenever possible, laboratories should collect pregnancy outcome data for pregnancies with initial screen-positive results, including the proportion of patients 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 laboratories with sufficient resources, complete pregnancy follow-up is recommended to determine the trisomy 21 DR. An alternative approach recognized by some regulatory agencies is to use epidemiological monitoring data as performance measures.[85] 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 initial and revised positive rates.

Tracking test failures

Although published data on test failures from screening programs are scarce, kit manufacturers provide information about acceptable sample types (eg, serum vs plasma), minimum sample volumes required, and conditions that can affect assay performance (eg, hemolysis, cross-reactivity). 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 because of a sampling error, are uncommon and likely resolvable by repeat testing. The rare occurrences of test failure requiring a second sample should be documented and tracked.

Reclassification of positive results

Laboratories should be aware of the potential problems associated with reclassifying screen-positive pregnancies as screen negative (ie, reclassifying a true positive as a false negative). Reclassification is primarily a concern for second-trimester screening and usually occurs when an LMP-dated pregnancy is later dated by ultrasound and the difference in dating exceeds a set standard (eg, >10 day discrepancy). An ultrasound-confirmed gestational age before screening is optimal. The need to reclassify first-trimester screening results is infrequent because CRL measurements are often provided.

Other conditions associated with a high DS risk (screen positive)

In both first and second trimesters, 95% to 98% of pregnancies with a positive screening result in an unaffected infant, corresponding to a PPV of 2% to 5%. There are many other causes of a positive result besides DS, including placental or maternal abnormalities, as well as fetal trisomy 18, trisomy 13, and steroid sulfatase deficiency (leading to low uE3 production). Additional details on conditions frequently detected in the course of DS screening are provided in Supplement 12.

Variables affecting anticipated DR and FPR

Detection rates and associated false-positive rates vary not only by screening cut-off, but also by the age distribution of the patients tested, the gestational age at sampling, and the combination of markers chosen. The laboratory will need to consider a number of factors in establishing expectations in screening performance as measured by DR and FPR.

Section 2: NT in first trimester screening

Validation of NT measurements

Sonographer training and expertise are critical to high quality DS screening using NT. Ongoing review of sonographic images and other quality data, including quantitative measurements, is also important for continuous quality assurance. Sonographers should provide the laboratory with paired NT/CRL measurements from at least 30 pregnancies to validate appropriate reference data before clinical interpretations. This ensures that the 95% CI of the median NT MoM is between 0.9 to 1.1 MoM. If this is not possible, the laboratory can use existing medians representative of that sonographer (eg, those recommended by the training program that credentialed the sonographer or those used by the sonographer's practice). As a last resort, a published generic set of medians can be used. For all sonographers new to the laboratory, epidemiological monitoring should be performed as soon as adequate data are available. Detailed information on the collaboration between laboratories and sonographers is given in Supplement 13.

NT performance monitoring

NT monitoring

Although laboratories should monitor the NT data they receive from each sonographer (and recalculate medians when appropriate according to established protocols), identifying the causes for deviations from expected ranges for epidemiological monitoring and subsequent remedial actions should be managed by the sonographers or professional/credentialing/training organizations. Supplement 13 addresses aspects of NT performance monitoring in greater detail.

Departures from expected NT performance

If center- or sonographer-specific medians are used, they should be updated each year or when monitoring indicates that the median NT MoM for that sonographer is consistently outside the target range (0.9-1.1 MoM).

When epidemiological monitoring of sonographer NT measurements identifies a significant departure from expected (for the increase per week and the logarithmic standard deviation), the laboratory should carefully review the data collection and analyses. If departures persist, the laboratory should contact the sonographer to discuss possible explanations (eg, a referral sonographer who sees a higher proportion of elevated NT measurements, inferior ultrasound equipment, multiple sonographers reporting under a single code). The laboratory may also consider contacting organizations that oversee sonographer training in the United States (see Supplement 13) to discuss possible sources of variation. If the discrepancy persists, the laboratory may choose to not accept prenatal samples from that sonographer. Laboratories should have a written plan to address performance expectations and how sonographer performance issues will be addressed should they arise.

Combining First and Second-Trimester Markers

Integrated screening

Integrated screening is a 2-stage process that combines the most informative DS markers from the first and second trimesters into a single risk assessment.[55] This can be done using first- and second-trimester serum markers only (serum integrated test) or serum markers in combination with first-trimester serum and ultrasound measurements (full integrated test). Because final screening results are only available in the early second trimester, the diagnostic follow-up for screen-positive patients is usually amniocentesis. Laboratories offering integrated screening should be prepared to have the first-trimester sample and NT measurement collected earlier in gestation and establish protocols for dealing with the absence of a second-trimester sample or its late arrival. Detailed methods for integrated screening are given in Supplement 14.

Sequential/stepwise screening (NT measurements available)

Sequential (stepwise) screening incorporates aspects of first- and second-trimester screening in a 2-step strategy to leverage the advantages of each (early diagnosis of DS and highest screening performance, respectively). First-trimester NT measurement is required for sequential screening, and follow-up CVS should be available for patients undergoing sequential testing. To maintain an acceptable balance between DR and FPR, only pregnancies with very high first-trimester DS risks (eg, >1:25 or >1:50) should be offered diagnostic testing in the first trimester. More than half of DS pregnancies occur in this group, at a corresponding FPR of 0.5%, or lower.[86] A second-trimester blood sample is obtained from the remaining patients (99.5%) to test the 4 additional markers, and the final risk is calculated using information from both trimesters.

Variations of the sequential test exist. Free beta-hCG or hCG is often measured in the first-trimester sample to improve performance. The interim first-trimester risk may, or may not, be reported to all patients; the laboratory should consider the risks and benefits of this strategy in developing

their plan for implementation (eg, patients receiving the first trimester risk may not complete second-trimester testing or may unnecessarily pursue diagnostic testing).

Contingent screening (NT measurements available)

In contingent screening, first-trimester results are divided into 3 outcomes: screen-positive, screen-negative, and intermediate/pending risk. Only patients with intermediate risks proceed to second-trimester testing and receive an integrated risk. This strategy allows for an early DS diagnosis in the small high-risk group (screen positives) while providing early reassurance to the large low-risk group (screen negatives). Contingent screening achieves its higher performance by subjecting only this intermediate risk group to additional testing while simplifying the patient experience for all others. Contingent screening is more complex for the laboratory to implement because it provides multiple risk estimates to many patients and leaves open the question of second-trimester screening for open neural tube defects. Formal pilot studies may be warranted before routine implementation.

Section 3: Incorporating cfDNA testing into serum screening

cfDNA as a secondary screening test

The high screening performance of cfDNA, with sensitivity and specificity for Down syndrome >99%, has led to its use as a secondary screening test in the evaluation of DS positive serum screen tests. The rationale for this approach is that the majority of positive tests with serum screening represent false positives. Nearly all true positives found by serum screening will also test positive with cfDNA, and the vast majority of false positives from serum screening will be identified as such. False positives, although rare, do occur with cfDNA, and it is therefore recommended that positive cfDNA tests be verified with diagnostic testing.

Contingent and reflex testing models

Two models merging combined testing with cfDNA have been clinically validated and implemented. Both start with combined testing and a lower risk cut-off that increases both the FPR and DR. In the "contingent" or "recall" model, the laboratory interprets results as high risk (eg, >1:150 for DS) and moderate risk (eg, 1:150 to 1:800) in which the 2 cut-off levels can be set as desired.[87] All patients receive results of the first trimester combined serum/ultrasound screening. Those in the higher risk category are counseled and offered the choice of invasive testing, cfDNA testing, or no further testing. The moderate-risk group is also counseled and offered cfDNA testing or no further testing. All high- and moderate-risk patients would return to the clinic for counseling, consent, and, if cfDNA is chosen, a new blood sample. The performance of screening cannot easily be modeled because the overall DR and FPR are dependent on patient choices (eg, amniocentesis vs cfDNA testing). In the "reflex" model, a blood sample is collected for screening, and a second sample is retained for cfDNA testing[88,89] to be used only if the first-trimester combined test reveals an increased risk (eg, >1:250). A single report is generated with the results of all tests performed. Positive cfDNA results will be found in a small number of patients, whereas the vast majority will receive a screen-negative result.

There are benefits and limitations to each of these approaches. The contingent model provides more options than the reflex, but far more patients are provided with an initial positive screening result, and more invasive tests are likely to be performed. The failure of patients to return for counseling or to complete the second sample collection poses a logistical complication in the contingent scheme. With reflex testing, there may be added costs for collecting, processing, and storing many samples for cfDNA that are never utilized. Overall, the 2 methods had approximately equal DR for trisomy 21 at similar costs, but contingent testing had far more patients who screened positive (100-fold) and subsequently offered invasive testing (20-fold). Both protocols have methods to handle cfDNA test failures, and each can detect both trisomy 18 and 13.[88,90] For both models, the DR for the common trisomies can be in the range of 91% to 95%. Patients and providers have found both contingent[14] and reflex[91] approaches to be acceptable, allowing the screening laboratory discretion in choosing which to implement.

cfDNA as a primary screen

Screening programs must weigh the pros and cons when considering a move from primary screening based on maternal serum/ultrasound findings (eg, 1st trimester combined, 2nd trimester) to primary prenatal cfDNA screening. On one hand, cfDNA screening for DS has a very low FPR and, most importantly, a very high DR.[40] In addition, the very low false-negative rate may offset the worry of a follow-up invasive procedure and make cfDNA more attractive to patients. The ability to reveal fetal sex early in pregnancy has also driven patient interest. However, the cost of cfDNA screening has been higher than serum screening and may not be as well covered by insurance, raising patient access concerns. In 2022, a systematic review of evidence resulted in evidence-based guidelines by ACMG.[92] The research group strongly recommended prenatal cfDNA screening for the general population over traditional methods in both singleton and twin pregnancies for trisomies 21, 18, and 13, as well as sex chromosome abnormalities. Finally, some programs, especially those outside of the United States, have chosen to maintain the first trimester ultrasound because it provides other useful information, including reliable and early pregnancy dating, visualization of structural anomalies, identification of multiple gestations, molar pregnancies, and fetal death.

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Conflict of Interest

All workgroup members receive salary for providing clinical services that may be relevant to the content of this document in either the laboratory or patient care setting at their listed affiliations. The following workgroup members have additional conflicts of interest: Glenn E. Palomaki and Geralyn Messerlian receive remuneration from UpToDate for prenatal screening educational programs. Robert G. Best's employer offers prenatal screening. All other authors declare no conflicts of interest.

Additional Information

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Supplemental Material

Prenatal screening for trisomy 21 (Down syndrome) using first- and second-trimester biochemistry and ultrasound measurements: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

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Supplement S1 – Other Existing Serum Screening Guidelines

Several relevant clinical guidelines have been published. These may differ in their focus compared to these ACMG Laboratory Technical Standards and Guidelines, but their review by laboratory directors may prove beneficial.

American College of Obstetricians and Gynecologists (ACOG)

The 2020 ACOG Practice Bulletin entitled Screening for Fetal Chromosomal Abnormalities,[1] focuses on screening for fetal chromosomal abnormalities and include recommendations for offering screening in the first and/or second trimesters using ultrasound, serum analytes and/or cfDNA tests. Also included are recommendations for the types of information available for patients, interpreting results, residual risks, interpreting cfDNA test results, first and second trimester ultrasound markers and dating, recommendations for twin pregnancies and risks associated with abnormal screening results.

Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG)

The SOCG/CCMG Clinical Practice Guideline for Prenatal Screening for Fetal Aneuploidy in Singleton Pregnancies[2] guidelines were reaffirmed in 2017 and include recommendations for screening in the first and/or second trimesters, ultrasound markers and dating, as well as analyte combinations.

Ontario Prenatal Screening Program

Standardized Procedural Practices – These 2012 guidelines cover prenatal screening for both Down syndrome and open neural tube defects.[3] Both first and second trimester serum/ ultrasound screening are covered, as well as serum integrated screening.

American College of Medical Genetics and Genomics (ACMG)

These 2007 Professional Practice Guidelines[4] focus only on first trimester ultrasound and biochemical testing for Down syndrome and trisomy 18 as well as the identification of cardiac and other anomalies.

American College of Medical Genetics and Genomics (ACMG)

These 2009 Professional Practice Guidelines[5] include both first trimester and second trimester screening for Down syndrome and second trimester screening for open neural tube defects. Guidelines for neural tube defect screening were updated in 2020 as a separate document from Down syndrome screening.[6]

Supplement S2 – Requisition Forms and Result Report Contents

Requisition Forms

For reliable interpretation, laboratories should collect pretest clinical information that includes basic required demographic information consistent with recommendations in the ACMG Standards and Guidelines for Clinical Laboratories[7] to include the following for prenatal serum screening:

a. Gestational age and its method of determination
b. Maternal weight (pounds or kilograms)
c. Maternal race/ethnicity. In the US, this can include two questions:

    Race (can choose more than one): White, Black, Asian, Pacific Islander, American Indian)

    Ethnicity: Hispanic/Latino
d. Number of fetuses
e. Family history of Down syndrome or neural tube defect (previous affected pregnancy)
f. NT measurement in mm (first trimester)
g. Crown-rump length (CRL) measurement in mm (first trimester)
h. Identification of sonographer and/or sonographer's center
i. Cigarette smoking status (Yes/No)
j. The laboratory may also choose to collect information regarding:
    i. Presence of insulin-dependent diabetes before pregnancy
    ii. Assisted reproductive techniques (eg, in vitro fertilization, date of embryo transfer, and age of egg donor if applicable)

When information is not provided, the report should indicate what information was missing and what was used in the interpretation. In some cases, including information on the report about the potential impact of the missing information may be warranted (eg, maternal weight); in other cases, full interpretation may not be possible (eg, missing maternal age or missing gestational

age).

Results reporting

Final reports of test results should be clear to a non-geneticist professional and must include (as appropriate):

a. Patient's name, date of birth, and other unique identifiers;
b. Name of referring physician/health center to receive the report;
c. The test that was ordered;
d. Type of specimen;
e. Date when sample was obtained;
f. Laboratory accession number(s) that uniquely identifies the sample;
g. Demographic and pregnancy-related information used in the interpretation (eg, LMP, ultrasound dating results (eg, from biparietal diameter (BPD), crown-rump length (CRL)), estimated gestational age, maternal weight, smoking status;
h. Nuchal translucency (NT) measurement and interpretive units (eg, NT in mm, NT converted to MoM);
i. Sonographer's name or ID (eg, credential number);
j. Analytic results in both mass units (eg, ng/mL) and interpretive units (eg, MoM) on which all adjustments/corrections have been performed;
k. Clinical interpretation, including the patient-specific risk for Down syndrome (DS), whether that risk is considered 'screen positive' or 'screen negative', and the risk cut-off level.

Supplement S3 – Choice of hCG in the First Trimester

The choice of which hCG form to measure in the first trimester depends on the markers' performance at each week of gestation.[8] A tabulation showing the DR and FPR at 11, 12 and 13 weeks' gestation by marker combination is shown in Table 1 of the 2009 ACMG Technical Standards and Guidelines.[9] Before 11 weeks of gestation, free beta-hCG is discriminatory between affected and unaffected pregnancies, but hCG is not. Between 11-12 weeks, free beta-hCG is univariately a more discriminatory marker than hCG. When combined with maternal age, NT, and PAPP-A, free beta-hCG performs better than hCG (2-3% higher detection at a fixed FPR) at 11 weeks. At 13 weeks, hCG may perform slightly better than free beta-hCG (1-2% higher DR at a fixed FPR); however, the instability of the intact hCG molecule can negatively impact screening performance based on free beta-hCG levels under certain conditions.[10] A small amount of dissociation of intact hCG results in significantly elevated free beta-hCG subunits over its relatively low baseline concentration. This phenomenon is exacerbated by temperature and humidity,[11] which can be mitigated using dried blood spots where the free beta-hCG subunit is more stable,[12] but this introduces variability due to the relatively low concentration of the free beta-hCG subunit in maternal serum. A limited number of US laboratories provide testing using free beta-hCG while such testing is common in Europe and elsewhere. When measuring free beta in serum, samples must be protected from high temperatures (eg, cool packs with overnight shipment in the summertime).

Supplement S4 – Screening Performance

The DR and FPR vary depending on the combination of serum analytes used, the DS risk cut-off level chosen, and the distribution of maternal ages in the tested population. Published studies are available that compare various marker combinations and risk cut-offs. Table 2 in the manuscript contains performance estimates for selected first and second trimester DS screening protocols, based on published parameters,[8,13-18] and should be used only as a guide for initial comparisons.

The positive predictive value (PPV) and negative predictive value (NPV) of testing in the target population measure the ability of the test to give accurate clinical information. The PPV is the percentage of positive test results that correctly identifies pregnancies with [number of true positives/(true positives + false positives) x 100]. The PPV can also be expressed as odds and is sometimes referred to as the odds of being affected given a positive result (OAPR). For example, consider a second trimester quadruple test with a DR of 80% and a FPR of 5%. The test is applied to a general pregnancy population where the term prevalence of DS is 1:500. Among 50,000 pregnancies tested, 100 cases of DS are expected, and 80 of those will be detected. Among the remaining 49,900 unaffected pregnancies, there will be approximately 2,500 false positive results. The PPV is, therefore, 80:2,580 or 1:32 or about 3%.

The negative predictive value (NPV) is the percentage of negative tests that correctly identify unaffected pregnancies [number of true negatives/(true negatives + false negatives) x 100]. Because the prevalence of DS in a screening population is low (eg, 1:500 at term in the general pregnancy population in the US), the NPV is generally not computed or reported. In this example, the NPV prior to testing is 49,900:100 or 499:1 or 99.8%). After testing, there are 47,400 unaffected pregnancies with negative results along with 20 affected pregnancies. The NPV after testing is 99.95% (47,400 true negative to 20 false negative or 2,370:1). This

represents about a four to five-fold reduction in risk.

Supplement S5 – Standards and Calibration

- AFP (alpha-fetoprotein) assays 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, and conversion factors are manufacturer specific.
- uE3 (unconjugated estriol) assays are typically calibrated in mass units (ng/mL) or the International System of Units (nmol/L). The conversion factor is 1 ng/mL = 3.467 nmol/L. Traceability of the calibrator is possible through the International Organization for Standards (ISO 17511).
- hCG (intact or total human chorionic gonadotropin) assays are commonly calibrated in mIU/mL using the World Health Organization 5th International Standard. Results can be reported as IU/mL (eg, 3.2) or mIU/mL (eg, 3200).
- DIA (dimeric inhibin-A) assays are standardized against the First International Standard for Inhibin (Code 91/624) and results can be reported in pg/mL or IU/mL. Commercially available kits provide calibrators and specific calibration protocols.
- Free beta-hCG assays are also calibrated using the First International Reference Preparation (equivalent to the Third International Standard) and results are reported in mIU/mL or ng/mL.
- PAPP-A (pregnancy-associated plasma protein A) assays In the US tend to report in mass units (500 ng/mL) or equivalent (50 ug/dL). Elsewhere, results are reported in international units (mIU/mL) or equivalent (mIU/L). There is no fixed factor to reliably convert mass units to international units. One publication directly compared the PerkinElmer assay (measured in IU) with Beckman Coulter (measured in ng) which found that the relationship across the range of results was reasonably constant.[19]

Supplement S6 – 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: knowing whether pilot trials have been undertaken and if so, what the results were; establishing or adopting quality assurance processes that monitor the effectiveness of the laboratory's ongoing testing activities; understanding possible adverse health or psychosocial consequences of testing; describing what follow-up testing or interventions might be reasonable in persons with positive test results; and understanding what is known about the financial costs and economic benefits of testing.

Supplement S7 – Determination of Medians for Serum Screening

Normative data

Values obtained from different manufacturers or different lots from the same manufacturer can demonstrate systematic proportional or non-proportional bias. The laboratory should establish internal normative data or, at a minimum, demonstrate that data obtained from another source are appropriate for its screened population.

Sample size

Ideally, 100 samples for each completed gestational week (15 through 20 weeks in the second trimester and 11 through 13 completed weeks in the first trimester; samples beyond 20 weeks or as early as 10 weeks can be included if available) would be used to calculate median values. Because these analytes are relatively stable (with the exception of free beta-hCG subunit), it may be possible to use stored frozen specimens. A consecutive series of fresh samples may also be appropriate. It is not necessary that all samples be obtained from unaffected singleton pregnancies as affected pregnancies are uncommon. Using regression analysis, fewer samples (eg, 300 spread over the 11-13 completed week period) can be used to establish reasonable medians.[20]

Computing medians

"Smoothing" the observed median values by weighted regression analyses provides more reliable and accurate medians. Appropriate models for each of the analytes can be found in the literature. In the second trimester, AFP and uE3 fit a logarithmic linear model, intact hCG and the free beta subunit of hCG an exponential model, and DIA a log cubic or quadratic model. In the first trimester, all analytes fit a log-linear model between 11 and 13 weeks' gestation.[21] The use of median values specific to each gestational day further improves screening performance and is strongly encouraged. For some laboratories, it may be necessary to establish median

values earlier than 11 weeks' gestation (see section on *Gestational Age*). If so, actual observed data are required, as extrapolation of the above models may not provide accurate results.

Expected change in medians by gestation

Between 15-20 weeks' gestation, AFP and uE3 levels increase by between 10-15% and 20-25%, respectively. Levels of hCG and free beta-hCG decrease by about 25% from 15-17 weeks and more gradually from 18-20 weeks. Measurements of DIA show the smallest gestational age effect, varying little over the 15–20-week window. They are slightly lower at about 17 weeks; the 15- and 19-week medians are about 10% higher.[22]

In the first trimester, PAPP-A levels increase by about 40-45% per week between 11-13 completed weeks' gestation.[21] Before 11 completed weeks, PAPP-A levels increase at a higher rate. Levels of free beta-hCG, hCG, h-hCG, and DIA decrease by about 20-40% from 11-13 completed weeks.[13,21,23]

Supplement S8 – Gestational Age Determination

Methods of assessing gestational age

Biparietal diameter (BPD) measurements are preferred as they represent unbiased estimates of gestational age in DS pregnancies, and they also improve performance for open neural tube defects (ONTD) screening. First trimester crown rump length CRL measurements provide an unbiased estimate of gestational age but do not improve ONTD screening. Gestational age based on composite second trimester measurements is often the only estimate available and can be used in place of dating by LMP.

First-trimester screening using NT and biochemical measurements are nearly always interpreted based on a CRL estimate of gestational age. The sonographer may report both a CRL (in mm) and an associated gestational age (in weeks + days, or decimal weeks). Note that not all sonographers use the same reference data to convert CRL to gestational age, which may lead to confusion [see Supplement S10]. The laboratory should report their conversion method to participating sonographers to avoid collecting samples at an unusable gestational age.

Gestational age measurements

Gestational age may be expressed in completed weeks (15 weeks, 5 days is 15 completed weeks). Expressing results in rounded weeks (12 weeks, 5 days is 13 completed weeks) is not recommended. Screening performance is significantly improved by expressing gestational age as weeks and days or decimal weeks (11 weeks, 2 days is 11.3 weeks).

Dating method to use

The most common method for determining gestational age is dating by the first day of the last menstrual period (LMP). In the first trimester, the most common ultrasound method for

determining gestational age is measurement of crown rump length (CRL). CRL measurements at 11-13 completed weeks' gestation (or earlier) provide an accurate estimate of gestational age to within seven days.[24] In the second trimester, ultrasound dating is accurate to within 10 days, but some measurements (eg, shortened femur and humeral length) are associated with the presence of DS, and their use (alone or in combination with other ultrasound measurements) reduces screening performance. Biparietal diameter (BPD) is not altered in DS, and it is also the recommended dating metric in screening for open neural tube defects. If available, ultrasound dating using CRL or BPD improves both the DR and FPR when compared to LMP dating.

Incorporating dating method

The dating method for gestational age can be applied to risk interpretations in two ways. First, separate medians can be calculated for pregnancies dated by LMP and by ultrasound measurements. In general, the association (slope) will be stronger (steeper) when using ultrasound measurements. More commonly, separate population parameters can be utilized in determining DS risk.

Because NT is measured early in pregnancy, first-trimester screening (and most integrated screening) is usually based on CRL measurements, which provide a reliable estimate of gestational age. This measurement is unbiased in both DS and unaffected pregnancies.[25] Given that nearly all gestational age estimates are based on ultrasound measurements, programs will likely have only one set of medians for each marker and use only one set of parameters.

Supplement S9 – Factors in Adjusting MoM Levels for Serum Screening

The interpretation can be refined with additional patient demographics and pregnancy-related information. These factors are less critical than gestational age but can improve screening performance by optimizing the interpretation.

Maternal weight

On average, serum concentrations of measured analytes are higher in patients weighing less than average and lower in patients weighing more. Adjusting for maternal weight only minimally improves screening for DS,[26] but offers benefits in other areas. AFP levels should be adjusted for maternal weight when screening for ONTD, and multiple analytes should be adjusted for when screening for trisomy 18. The relationship between PAPP-A and maternal weight is stronger than for any other marker.[27] Laboratories should utilize published weight adjustment formulas only until in-house data are collected and new laboratory-specific formulas are derived.[26]

Maternal race

Correction for maternal race/ethnicity should be made for AFP and hCG measurements. For both, levels in Black (African American) patients are about 10-15% higher than in White patients.[28] When the laboratory director determines that sufficient data are available, a separate set of medians should be determined for each group, otherwise a correction factor from the published literature may be applied to the MoM when screening those pregnancies. Effects of race and ethnicity are better defined for PAPP-A, hCG, and free beta-hCG, with clear increases in PAPP-A levels in Blacks of 25% or more[28-30] and lesser changes for Asian- and Hispanic-Americans. DIA levels are 8% lower in Blacks compared to Whites[31] and should be accounted for in DS interpretations. Laboratory directors should periodically review the literature on these topics and should consider emerging medical literature around the use of race in clinical care

and how it may sometimes be incorporated inappropriately during medical testing. Recent literature[32] concludes that these differences are not needed. However, a follow-up study[33] confirms the original findings.

Maternal insulin-dependent diabetes mellitus (IDDM)

The impact of IDDM on AFP levels was initially reported to be a 25% reduction in AFP levels,[34,35] and many laboratories corrected AFP for maternal IDDM by using an adjustment factor for AFP MoM levels; however, more recent data are conflicting.[36-38] Other second trimester analytes are less influenced by IDDM, but the laboratory director may want to adjust these as well by factors available in the literature.[38] There is no consensus on whether to apply this correction to patients with gestational diabetes. IDDM effects for the first trimester biochemical markers are not well described.[39] Laboratory directors should periodically review the literature on these topics and the decision of whether to make adjustments should be documented.

Maternal cigarette smoking

In patients who report smoking tobacco, first trimester levels of free beta-hCG and PAPP-A are reduced by about 10% and 15%-20%, respectively.[40] In the second trimester, patients who smoke have lower estriol (by 4%) and hCG (by 16%), but increased AFP (by 3%) and DIA (by 39%) relative to non-smokers.[41,42] These effects are not dependent on the number of cigarettes smoked. The birth prevalence of DS does not seem to be influenced by smoking status.[43] At this time, laboratories should consider adding a simple yes/no question concerning cigarette smoking status to their requisition slip. Methods to incorporate such information in a DS screening program and the expected results have been published[40] and can be used as a guide.

Correction for smoking is recommended for both first and second trimester markers. In the second trimester, correction is needed to avoid unnecessarily high screen positive rates for DS when using the Quad test. In the first trimester, correction is needed to avoid unnecessarily high screen positive rates for trisomy 18 when using the Combined or Integrated tests.[42] Accounting for smoking status when interpreting risks is up to the discretion of the laboratory director and may depend on the specific software used for risk analysis.

Serum screening marker levels in patients who report that they have recently quit smoking, often in conjunction with trying to or achieving pregnancy, are similar to those in patients who do not smoke. Patients who have recently quit smoking can be treated as non-smokers for risk analysis.[44]

Assisted reproductive techniques (ART)

First-trimester serum markers levels have been studied in pregnancies conceived through various types of assisted reproductive technologies ART, primarily *in vitro* fertilization (IVF), but a consensus has not yet emerged. Laboratories and software manufacturers have discretion to implement ART adjustments and should periodically review the literature on this topic.[45-54] In the second trimester, there is a clear effect of pregnancies achieved by IVF on serum screening markers. Maternal serum levels of uE3 decreased by about 10%, while hCG and DIA increased by about 10% in IVF relative to spontaneous pregnancies. Without adjustment, this would lead to an inappropriately high DS screen positive rate in IVF pregnancies.[53,55]

Part of the challenge in implementing correction for IVF is being able to collect accurate information from providers. An obstetrician may not be aware that the pregnancy resulted from ART or may fail to provide treatment details. It is optimal for screening to know the specific ART procedure used and particularly whether egg donation or surrogacy is involved. With egg

donation, the age of the donor is substituted for maternal age when calculating the *a priori* risk.[56]

Use of multiple correction factors to calculate the MoM

In many situations, multiple adjustments to the analytic result (expressed in MoM) may be warranted. Although data are sparse, in the absence of additional data, programs generally make the assumption that the effects are independent. For example, although most data for cigarette smoking were initially derived from White patients, the assumption is might be made that a similar effect will be seen in other populations of patients until the laboratory accumulates sufficient data to determine otherwise.

Supplement S10 – Variables that Impact either the Prior Risk or the Screening Parameters

Time of testing

For second trimester DS screening, the optimal time for sample collection is 16-18 completed weeks. Although reliable interpretation of risk using the triple or quadruple test might be possible at 14 weeks, this practice is discouraged because of the associated poor open spina bifida screening performance. Although reliable risks might be possible after 18 weeks' gestation, screening this late in pregnancy limits the options available when a positive test result be found.

The optimal time in the first trimester for DS screening based on serum testing alone is 11 completed weeks, or even earlier if using free beta hCG.[8,14] Before 11 weeks, free beta-hCG is useful but hCG is not.[57,58] PAPP-A measurements are most discriminatory at 9 weeks' gestation. However, professional sonographer organizations suggest that NT measurements be done between 11-13 completed weeks. The gestational age window for NT measurements will likely define the window for biochemical testing, although some suggest collecting a blood sample several weeks before the NT measurement is performed. If offering testing at 10 weeks' gestation or earlier, laboratories should increase the number of samples used to compute reliable medians within each gestational week where an interpretation will be provided.

Multiple gestation

In the second trimester, DS screening in twin pregnancies is less effective than for singleton pregnancies. Algorithms for assigning a "pseudo risk" have been published. When pseudo risks for twins are reported, the limitations should be included in the report. Such algorithms may also consider zygosity by using chorionicity as a surrogate.

In the first trimester, DS screening in twin pregnancies is also less effective than for singleton

pregnancies. Serum marker levels in twin pregnancies are pregnancy-specific. In contrast, first trimester NT measurements are fetal-specific. Algorithms for assigning a pregnancy-specific risk when combining NT and biochemistry have been published.[59] Such algorithms can also take into account the zygosity of twins by observing chorionicity. Both in theory and practice, first trimester screening tests in twin pregnancies that include NT measurements have better performance than second trimester screening for DS.[60]

Family history

Family history of DS 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 DS risk estimate using published algorithms.[61] It may be necessary to inquire for more details on any reported history as the risk of aneuploidy for the current pregnancy is not increased by prior aneuploidy in distant relatives.

Computation of risks

Risks can be expressed relative to DS prevalence in either the trimester at which the sample is taken (1st or 2nd), or at term. Calculation of the 1st trimester risk is achieved by multiplying the term risk by 1.75 (or alternatively multiplying the denominator of the term risk by 0.57), and in the 2nd trimester, multiplying by 1.3 (or the denominator by 0.77). The lower risks at term compared to 1st and 2nd trimester are due to the likelihood of pregnancy loss. For example, working from the denominator of the risk, if the risk to a 35-year-old woman of having an infant with DS at full term is 1:350, the corresponding (and equivalent) second trimester risk is 1:350 x 0.77 or 1:270. The factor of 0.77 represents a reliable estimate of the proportion of DS fetuses that survive from the early second trimester to term.[62] For first trimester screening, risks can be

expressed as risks in the first trimester, or at term (rarely in the second trimester).[62] For example, for the patient above with a risk at full term of 1:350, the corresponding (and equivalent) first-trimester risk is 1:350 x 0.57 or 1:200. The factor of 0.57 represents a reliable estimate of the proportion of fetuses with DS that survive from the late first trimester to term.[62] Lower estimates for this proportion have also been published.[58]

Supplement S11 – Appropriate Recommendations for Follow-up of Screen Positive Results

Genetic counseling

All patients with a screen positive result should receive genetic counseling to understand the test result implications and options for follow-up care. This can be provided by the physician or a specifically trained genetic counselor. A referral to a genetics specialist might be necessary when there is a complicated family history.

Invasive procedures and diagnostic testing

Prenatal chromosome analysis is generally available through two invasive testing options; amniocentesis or CVS. Beginning at 15 weeks, amniocentesis is generally offered as a diagnostic test to determine the chromosomal status of the fetus. CVS is usually offered between 10 weeks 0 days and 12 weeks 6 days of gestation although many physicians perform CVS up to 13 weeks 6 days.[63] Using the collected material, the laboratory may perform karyotyping or molecular-based testing to identify common trisomies.

Second tier screening with cfDNA

The use of prenatal cfDNA screening has become increasingly common because of the very high sensitivity and specificity of this modality for screening for DS. False positives and false negative both are rare, but care should be taken to confirm positive cfDNA findings.

Subsequent ultrasound in the second trimester

Some programs may continue to utilize ultrasound “soft markers” (eg, shortened long bone measurements) to modify the DS risk assigned using the biochemistry results and maternal age. In the absence of finding an ultrasound marker, the DS risk is considered lower than reported by the serum screen, up to 2-fold. While this practice can reduce the number of pregnancies

referred for amniocentesis, it will also reduce the program's DS detection rate. Screening programs should be aware of the advantages and disadvantages of this practice.[64]

Supplement S12 – Other conditions Identified by a Down syndrome screening program

Trisomy 18 (Edwards syndrome)

Trisomy 18 results in a complex constellation of structural and neurological anomalies (Edwards syndrome) with higher morbidity, spontaneous loss rate and neonatal mortality compared with trisomy 21. In the absence of prenatal diagnosis and termination, the age-specific birth prevalence of trisomy 18 is much lower than for trisomy 21 (about 1:4,000 for trisomy 18 compared with 1:500 for trisomy 21 in the first trimester). An estimated 65% of trisomy 18 fetuses are viable in the second trimester, while 72% in the first trimester will be spontaneously lost by term.[65] possibility of cesarean section in patients with an undiagnosed fetal trisomy may result in unnecessary maternal morbidity. Although it would be difficult to justify a screening program aimed solely at identifying trisomy 18, such secondary target screening is a common addition to maternal serum screening programs for trisomy 21.

The pattern of second trimester markers is different in trisomy 18 compared to trisomy 21, so a separate algorithm is required. Published algorithms are available that estimate an individual pregnancy's risk of trisomy 18.[66] Usually the FPR is kept at 0.3% or lower, and the corresponding DR using maternal age in combination with AFP, uE3, and hCG measurements is about 70%. Maternal weight correction is a vital component of trisomy 18 screening. Unlike trisomy 21 screening, the marker pattern associated with trisomy 18 cannot be caused by incorrect gestational dating; therefore, re-dating the pregnancy is usually not informative. DIA is not useful when screening for trisomy 18.[67]

The pattern of first trimester markers is also different in trisomy 18 compared with trisomy 21. Algorithms are available that estimate an individual pregnancy's risk of trisomy 18.[68] In the first trimester, published data suggest that laboratories may also be able to detect at least 75% to 80% of trisomy 18 fetuses using maternal age in combination with NT, PAPP-A, and hCG

measurements at a false-positive rate of <1%.[68,69] Because of the high fetal loss rate for trisomy 18 pregnancies from the late first trimester to term, the true DR is difficult to reliably quantify.

Trisomy 13 (Patau syndrome)

Trisomy 13 is another autosomal trisomy that causes a different severe pattern of physical and neurological features with very high rates or prenatal and neonatal mortality. Patau syndrome has a much lower birth prevalence compared to trisomy 21 (about 1 per 7,000). The existing data (limited to intervention trials subject to ascertainment bias) indicate that the first trimester biochemical and ultrasound measurements in trisomy 13 pregnancies can be useful in identifying a high-risk group that could be offered diagnostic testing.[69] Programs can consider reporting risks for trisomy 13 as another secondary screening target but should be mindful that these risks may not be as reliable as those reported for trisomy 21 and the associated clinical utility is less certain. The risk cut-off chosen should be associated with a low screen-positive rate and a high PPV. In general, first trimester screening programs should consider adding a trisomy 18 screening protocol that will likely identify most of the trisomy 13 pregnancies.

Steroid sulfatase deficiency and other abnormalities

In a small proportion of pregnancies, second trimester uE3 measurements will be below the lower limit of assay sensitivity (<0.1 ng/mL or about 0.1 MoM) while the other markers are near normal. A causal explanation can be identified in the majority of cases (eg, preexisting fetal death). Some of these remaining pregnancies will be male fetuses with steroid sulfatase deficiency (OMIM 300747).[70,71] This deficiency manifests as X-linked ichthyosis (a mild to moderately severe skin disorder). Smith-Lemli-Opitz syndrome (SLOS - OMIM 270400) is also associated with extremely low uE3 (median = 0.21 MoM, on average), and modestly reduced levels of AFP and hCG (~0.7 MoM). Although an SLOS screening algorithm can be

implemented, its utility is questionable because of the prevalence in the second trimester, on the order of 1 in 100,000.[72] There is no consensus as to whether these disorders meet the criteria for prenatal screening and intervention. It is reasonable for a laboratory to choose not to routinely comment on these results. However, implementing a formal interpretation for SLOS does, incidentally, identify an array of abnormal outcomes.[72,73] In a study of 516,172 second trimester screens in California, 0.8% were screen positive only for SLOS.[73] Upon the recommendation to follow-up the screen positive result with ultrasound, it was determined that 1 in 8 (12%) of these had a fetal anomaly including an open neural tube defect, molar pregnancy, or other anatomical defect.

Supplement S13 – Collaborating with Sonographers; NT measurements

Proof of training, credentialing, or certification

Laboratories should try to obtain, and have the right to require, documentation of specialized training and successful submission of NT images (and associated data) that qualifies participating sonographers and their supervising physicians specifically for this purpose. Such documentation could consist of the sonographer/physician providing such information and/or by searching databases listing qualified sonographers (eg, Nuchal Translucency Quality Review (NTQR) program will cease review of NT at the end of 2024,[74] Fetal Medicine Foundation (FMF)).[75] If a sonographer cannot document their qualifications, the laboratory can choose not to accept the prenatal data for interpretation.

Providing NT and crown-rump length (CRL) data to the laboratory

At a minimum, the sonographer must report the date of scan, NT, and CRL measurement in mm, as well as the number of fetuses. In addition, the laboratory should have methods in place to identify each sonographer providing NT measurements. This may be a laboratory-specific code, or the code assigned by an NT training program (eg, NTQR). Sonographer initials (alone) are likely not unique. Additional data that may be useful include a center code, a supervisor code, and the estimated gestational age.

Conversion of CRL to gestational age

There is no universally accepted equation to convert CRL (in mm) to gestational age (in weeks + days, or decimal weeks). This can cause problems when determining whether an NT measurement was taken within the acceptable gestational age window. Most groups suggest obtaining an NT measurement between 11 + 0 and 13 + 6 (weeks + days). This range, when converted to CRL, is dependent on the equations used. Given that the CRL, and not the gestational age, is being measured, it is appropriate to use the CRL to define the screening

window. Equations commonly used by sonographers include one published in 1992 by Hadlock et al.[24] This yields CRL limits of 39 to 84 mm. Other conversions are also available.[76,77] In the United Kingdom, there is an effort to use a common methodology and conversion equation.[78]

The CRL or NT measurement is missing

If the CRL is missing, gestational age cannot be reliably estimated. As a result, it is not possible to confidently interpret either the maternal serum or NT measurements. Thus, the laboratory may consider the sample to be inadequate. The NT measurement might be initially missing but provided later (with an accompanying CRL). Thus, laboratories should have the capability of interpreting an NT measurement that arrives at a different time in gestation from the biochemistry sample, so long as that original sample and the NT measurement were collected within the specified gestational age window.

Establishing sonographer-specific, center-specific, or single set of medians for NT measurements

Some proponents of NT measurements such as the Fetal Medicine Foundation (FMF) argue that only a single "master" set of medians is necessary if sonographers are properly trained.[79] That is, all sonographers can be trained to measure NT in exactly the same way. This is the rationale for programs of the NTQR and the FMF. Although standardization of technique among sonographers is important, it may not be necessary (or possible) to achieve the uniform performance level required to allow use of only one universal set of medians. Indeed, at least two research trials have analyzed the NT data by both center-specific and sonographer-specific medians. In both, the use of a single master set of medians was associated with lower screening performance than with the use of sonographer-specific medians.[14,15] Laboratories should routinely analyze the NT results by sonographer to determine whether there are important differences. If so, it is recommended for them to use center-specific or sonographer-

specific medians. Laboratory software should at least be able to use center-specific medians. The use of a difference between expected and observed NT measurements (delta NT) has been suggested as an alternative to using the ratio of observed to expected NT measurements (NT MoM).[80]

Frequency of QA/epidemiological monitoring

The frequency of monitoring depends on numbers of NT measurements reported. If monitoring is performed with too few samples, large variations from the expected might be due to random chance. Alternatively, if monitoring is performed too infrequently, interpretations may not be of reasonable quality. Optimally, at least 50 NT observations should be evaluated quarterly (a rate of 200 per year). If too few samples are available for quarterly monitoring, then biannually is an acceptable alternative (100 per year). If fewer than 50 samples are available over a one-year time period, it may not be possible to assess the quality of NT measurements from that sonographer. Written laboratory guidelines must be in place for dealing with sonographers providing small numbers of observations. It may be possible to obtain data from sonographers who have sent NT measurements to other laboratories by the laboratory's participation in the NTQR program.[74,75] Another solution to address sonographers with a small number of scans is to combine individuals within a center who have similar results. In all instances, sonographers should receive routine feedback from the laboratory regarding number of pregnancies tested and the three epidemiological parameters.

Who should handle NT QA training and monitoring?

NT training

Screening laboratories will not usually have the expertise or resources to be involved in educating, training, and credentialing sonographers (see section on *Proof of Training, Credentialing, or Certification*). These activities should be handled by groups with special

expertise in prenatal ultrasound such as the Nuchal Translucency Quality Review Program or NTQR[73] and the Fetal Medicine Foundation (FMF).[74]

Performance guidelines for quality assurance (QA) and epidemiological monitoring

Laboratories should assess individual sonographer performance by monitoring (1) percent increase of NT measurements per week; (2) median NT MoM assigned, and (3) logarithmic standard deviation of NT MoM levels. This approach has been used successfully in the First and Second Trimester of Risk (FASTER) trial.[15] Reasonable target ranges for the three parameters are (1) 15-35%, (2) 0.90 to 1.10 MoM, and (3) standard deviations between 0.08-0.13.[81] Neither the percent per-week increase nor logarithmic standard deviation are under the direct control of the laboratory, but the laboratory can adjust median values when the median MoM for a given sonographer is outside the expected range. Laboratory directors should monitor the literature to update acceptable ranges for these epidemiological parameters.

Use of Additional Ultrasound Markers

Nasal bone

The identification of an absent nasal bone between 11 and 13 completed weeks' gestation is reported to be a useful marker for DS.[82] However, reliable nasal bone evaluation and measurement require additional training and demonstration of proficiency. Inclusion of nasal bone measurement is not a standard part of a routine DS screening in the United States but may be performed as a follow-up test for pregnancies that screen positive in the first trimester.[63,83,84] The screening software must be able to modify the test risk for this finding to provide information that a patient can use in making diagnostic test decisions.

Other markers

Some prenatal screening centers have expertise to measure other standardized measures such

as the fronto-axillary angle and Doppler flows across the tricuspid valve and ductus venosus.[85-89] Laboratories may choose to include these markers in their risk algorithms if sufficient data are available to allow combining the new results with existing serum biochemistry and the more common ultrasound markers such as NT.

Supplement S14 – Integrated Screening

Serum integrated screening (NT measurements not available)

For the serum integrated test, PAPP-A is measured in a blood sample obtained in the first trimester, but the result is not reported. Although PAPP-A is a strong marker, even when added to maternal age it is not sufficiently discriminatory to report those results. Instead, a second-trimester blood sample is obtained from the same woman, and additional markers (eg, AFP, uE3, free beta-hCG or hCG, and DIA) are measured. First-trimester PAPP-A measurements plus the four second-trimester analytes are then combined with maternal age to produce a single patient-specific risk in the second trimester. At a fixed 5% FPR, the DR is about 89% (if the first-trimester sample is obtained at 10 weeks' gestation).[14] This is similar to the screening performance of the first-trimester "Combined Test" (Table 1 – main manuscript), which requires NT measurements. The serum integrated test can be offered to patients who present for prenatal care in the first trimester for whom a reliable NT measurement cannot be obtained.

The laboratory should decide the gestational age range for which it will accept samples for serum integrated screening. Because NT measurements are not involved, the lower gestational age limit for the first-trimester sample collection can be earlier than 11 weeks' gestation, a time when PAPP-A measurements are more discriminatory.[90] However, laboratories would need to obtain appropriate DS risk equations and develop reliable median levels before offering the test clinically. Another issue is whether to accept samples that are dated by LMP. The gestational dating method of choice for the serum integrated test is an ultrasound measurement of either first-trimester CRL or, less desirably, a second-trimester BPD. Because PAPP-A levels increase rapidly during the late first trimester, the use of LMP dating may result in an inappropriately high screen-positive rate and is not recommended.

When a serum integrated test has been ordered but the second-trimester serum sample is not

received promptly, the laboratory should contact the referring physician for further information to resolve the issue. If the expected sample is still not forthcoming, the laboratory can choose either to issue a report stating that the test result cannot be calculated or to retrieve the first-trimester serum sample from storage and perform further testing. A first-trimester test based only on maternal age, PAPP-A, and hCG (or free beta-hCG) measurements has an estimated DR of about 60% at a 5% false-positive rate.[14]

Full integrated test (NT measurements available)

For the full integrated test, an NT measurement collected within the appropriate gestational age range is included, along with the five serum markers. Together with maternal age, these are used to calculate a single patient-specific risk. At a fixed 5% FPR, the estimated DR is about 92%, with the first-trimester data collected at 12 weeks' gestation (Table 1). At a fixed 1% FPR, the corresponding DR is 83%.[14] Depending on the maternal age distribution of the population being screened, a 1 in 100 second-trimester risk cut-off will provide a DR of 85% to 90%, with a 1% to 2% false-positive rate.[19]

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