Fetal Laboratory Medicine: On the Frontier of Maternal–Fetal Medicine
Sharon M. Geaghan1*

BACKGROUND: Emerging antenatal interventions and care delivery to the fetus require diagnostic support, including laboratory technologies, appropriate methodologies, establishment of special algorithms, and interpretative guidelines for clinical decision-making.

CONTENT: Fetal diagnostic1 and therapeutic interventions vary in invasiveness and are associated with a spectrum of risks and benefits. Fetal laboratory assessments are well served by miniaturized diagnostic methods for blood analysis. Expedited turnaround times are mandatory to support invasive interventions such as cordocentesis and intrauterine transfusions. Health-associated reference intervals are required for fetal test interpretation. Normative fetal data obtained from negative fetal cordocenteses can be confirmed by physical examination results that confirm the health of neonates whose fetal data were collected. Strategies for assurance of sample integrity from cordocenteses and confirmation of fetal origin are described. After birth, definitive assessment of prenatal environmental and/or drug exposures to the fetus can be retrospectively assessed by analysis of meconium, hair, and other alternative matrices. A rapidly advancing technology for fetal assessment is the use of fetal laboratory diagnostic techniques that use cell free fetal DNA collected from maternal plasma.

SUMMARY: Developmental changes in fetal biochemical and hematologic parameters in health and disease are continually delineated by analysis of our collective outcome-based experience. Noninvasive technologies for fetal evaluation are realizing the promise of lower risk yet robust diagnostics; examples include sampling free fetal DNA from maternal blood, and analysis of fetal products accessible at maternal sites. Application of diagnostic technologies for nonmedical purposes (e.g., sex selection) underscores the importance of ethical guidelines for new technology implementation.

Fetal laboratory medicine offers a pioneer opportunity for diagnostic testing, forging the frontier of maternal–fetal medicine. Clinical trends in antenatal technologies demand laboratory support and expertise, beginning with diagnostic sample procurement, establishment of healthy fetal reference values, strategies for confirmation of fetal specimen identity, fetal health assessment in the context of biochemical and hematologic development, alternative matrices for analysis, and maternal sampling for identification of fetal secretary products, and extend to guidance for future directions in noninvasive prenatal molecular diagnostics for fetal health assessment. The objective of this review is to evaluate the current scope and contributions of fetal laboratory testing. A systematic literature review was performed. Extensive electronic searches were carried out in the PubMed database for fetal laboratory testing. Articles were selected on the basis of study characteristics, quality, and results. Reference lists of articles obtained were searched for any further articles. There were no language restrictions.

Fetal Therapeutic Interventions

The spectrum of fetal therapeutic interventions ranges in degree of invasiveness from open fetal surgeries performed in utero to minimally invasive fetoscopic surgery, which allows for small incisions under sonographic guidance (Table 1). The field of fetal surgery was first developed on animal models approximately 3 decades ago at the University of California, San Francisco, by Dr. Michael Harrison. The first human open fetal surgery was completed by Dr. Harrison on a fetus with congenital hydronephrosis due to congenital urinary tract blockage. Obstruction to urinary flow can cause permanent renal damage and other sequelae, such as poor lung development, if untreated. A vesicos-
tomy (placement of a catheter into the bladder) was performed, allowing urine to pass until definitive revision of the obstruction postnatally (1).

Open fetal surgery begins with a hysterotomy, or uterine incision, performed under general anesthesia. Maternal safety is prioritized above surgical success and preterm birth avoidance. Fetal size and fragility prohibits surgery before 18 weeks gestation. The fetus is exposed and the surgery performed (Fig. 1). The fetus remains dependent on placental support and is returned to the uterus following surgery. Before uterine closure, amniotic fluid is replaced, and the abdominal wall closed. Antenatal surgical interventions may be an interim procedure, to allow further development and maturation in utero until a definitive postnatal surgery can be performed.

Following open fetal surgery, preterm birth is common and cesarean section is mandatory. These surgeries are high risk, and are still considered experimental (2). For prenatal diagnosis and management of bilateral hydronephrosis (the indication for the first fetal surgery), for example, very few fetuses will undergo prenatal surgery. Laboratory diagnostic tests were employed for case selection, including fetal measurements: (a) urine Na <100 mmol/L; (b) urine Cl <90 mmol/L; (c) urine osmolarity <210 mosmol; (d) renal sonographic evaluation; (e) amniotic fluid status; and (f) urine output at fetal bladder catheterization. More recently, other metrics have been shown to be perhaps more predictive of poor renal function (see later discussion). Other indications for fetal interventions include: thoracic space-occupying lesions; amniotic bands; chorangioma; cardiac malformations; congenital diaphragmatic hernia; sacroccygeal teratoma; and neural tube defect closure (3).

The field has expanded to include a range of antenatal surgical techniques: Fetendo, or minimally invasive fetoscopic surgery, is performed in utero under real-time ultrasonographic guidance, through minute uterine incisions or even without incisions, by using intrauterine ultrasonographic and endoscopic views. Clinical indications include: fetoscopic closure of spina bifida; atrial septostomy; aortic or pulmonary valvuloplasty; treatment of fetal bladder obstructions; and balloon treatments of tracheal occlusions associated with congenital diaphragmatic hernia (3).

Most compelling was a recent randomized trial of prenatal vs postnatal repair of myelomeningocele, in which the trial was stopped for substantially greater efficacy of prenatal surgery based on results at 12 months of age. Shunt placement rates were 40% in the prenatal surgery group, compared with 82% in the postnatal surgery group (P < 0.001). Significant improvement for composite mental development and motor function scores at 30 months and secondary outcomes of ambulation by 30 months and hindbrain herniation by 12 months were manifest (4). Maternal and fetal risks were increased (uterine dehiscence at delivery and preterm delivery rate).

### Table 1. Fetal therapeutic interventions (in order of increasing invasiveness) and clinical indications.a,b

<table>
<thead>
<tr>
<th>Antenatal intervention</th>
<th>Clinical indication(s)</th>
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<tbody>
<tr>
<td>Delivery of therapeutic drugs, antibiotics, or steroids to fetus by maternal administration</td>
<td>Thyroid hormone in cases of maternal thyroid dysfunction; antibiotics for intrauterine infection; and steroid administration for accelerated lung maturation and to reduce the incidence of respiratory distress and neonatal mortality in the setting of premature births, or for confirmed congenital adrenal hyperplasia</td>
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<tr>
<td>Intrauterine transfusion</td>
<td>Fetal anemia (e.g., Rh isoimmunization)</td>
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<tr>
<td>Surgery on fetal membranes</td>
<td>Amniotic band syndrome (release)</td>
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<tr>
<td>Placental surgery</td>
<td>For placental chorangioma, to prevent cardiac failure and hydrops</td>
</tr>
<tr>
<td>Laser ablation or photocoagulation of placental vasculature</td>
<td>IUGR or twin–twin transfusion syndrome due to placental vascular anastamoses between monochorionic twins</td>
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<tr>
<td>Urinary obstruction decompression by vesicoamniotic catheter placement/shunting</td>
<td>Congenital hydronephrosis (urinary obstruction), with adequate renal function and pulmonary immaturity such that delivery must be delayed</td>
</tr>
<tr>
<td>Fetoscopy (direct endoscopic visualization)</td>
<td>Percutaneous tracheal occlusion procedure for lung growth promotion in congenital diaphragmatic hernia; tissue biopsies; vascular access; diagnosis and treatment of urinary tract obstruction (see below); surgeries on placenta, membranes, cord</td>
</tr>
<tr>
<td>Open fetal surgery</td>
<td>Meningomyelocele repair; congenital diaphragmatic hernia repair; thoracic space-occupying lesion removal; cardiac malformation repair; lower urinary tract obstruction decompression; sacroccygeal teratoma resection</td>
</tr>
</tbody>
</table>

a Harrison et al. (1), Crombleholme et al. (2), Deprest et al. (3), Adzick et al. (4), Quintero et al. (5), Leviton et al. (6).
b Adapted with permission from Geaghan (94).
This randomized trial represents the most definitive study to date demonstrating the value of prenatal surgery for a clinical indication. The results of this trial demonstrate that certain fetal surgeries can be performed safely without laboratory support. Only the posterior aspect of the fetus is exposed in these repairs. Intraoperatively, maternal status is stable and placental circulation continues to support the fetus.

Other technologies include fetoscopically directed selective laser (photocoagulation) ablations of placental vascular communications for intrauterine growth retardation (IUGR) due to vascular anastomoses (communications between twin circulations) in monochorionic twin pregnancies. If successful, the pregnancy is thereafter functionally dichorionic (with independent circulations), and risk of serious sequelae of twin–twin transfusion syndrome (neurologic damage, morbidity, and mortality) is reduced. Improved neurological outcomes have been reported for twins treated at <26 weeks gestational age. Procedural risks include IUGR, twin deterioration, or even demise.

Minimally invasive modalities include therapeutic pharmaceuticals delivery via maternal administration and placental transport to the fetus, such as antibiotics for infectious diseases, steroid administration for acceleration of lung maturity to reduce respiratory distress syndrome and neonatal mortality in cases of threatened preterm birth, or thyroid medications in the setting of maternal thyroid disease. In many types of fetal intervention, the elegant mechanisms of placental circulation allow for compensation and maintenance of fetal homeostasis.

The spectrum of diagnostic laboratory support required for these interventions ranges from no testing (myelomeningocele repair) to pathologic tissue analysis (sacrococygeal teratoma resection), serial monitoring by urinalysis (lower urinary tract obstruction), and life-saving transfusion when required (e.g., laser photocoagulation of placental anastomoses in twin–twin transfusion syndrome).

**Fetal Diagnostic Technologies**

Fetal diagnostic modalities vary widely in invasiveness and associated risk to mother and fetus (Table 2). Umbilical cordocentesis, or ultrasound-guided percutaneous umbilical cord blood sampling (PUBS), is performed for fetal health assessment and for fetal disease management. Despite the growing number of applications for this procedure, cordocentesis is not without risk. The amount of blood sampled depends on the indication, but can be 1–3.5 mL, a substantial amount for a fetus. In a large series of 341 cases reported complications included: bradycardia (9.38%)
related to repeated and prolonged punctures; cord hematoma (1.47%) associated with puncture attempts targeting a free loop of cord; and fetal deaths (5.87%), 3 of which were directly related to the procedure (0.88%). Literature review and metanalysis of 4922 cases revealed similar rates (bradycardia 3.1%–11%; total fetal deaths 3.84%; fetal deaths directly related to procedure 0.98%) (8). Other complications, reported in the largest published series of 606 consecutive cases, include spontaneous abortion (0.8%), growth retardation (8%), in utero death (1.1%), and premature birth (5%) (9). Because the complication rate is much higher if the procedure exceeds 10 min or if more than 3 punctures are attempted, practice guidelines recommend that attempts should not exceed 10 min, and a maximum of 2 punctures be performed at 1 session. Overall, the procedure failure rate (no sample procured) reported in the literature is 1.68%–8.71% (8). A single umbilical artery (rather than the expected 2-artery, 1-vein cord) precludes this procedure, because there is less opportunity for collateral circulation in the event of spasm or damage.

Therapeutic PUBS procedures, such as in utero transfusions, have reportedly higher complication rates (8). Intrauterine fetal transfusions require provision of blood counts in real time, at the point of care, to guide treatment. Our institution has a dedicated small footprint instrument mounted on a wheeled, stable

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### Table 2. Antenatal diagnostic laboratory technologies: clinical applications, associated risks and benefits.

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Clinical Application</th>
<th>Risks</th>
<th>Benefits</th>
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<tbody>
<tr>
<td>Amniocentesis</td>
<td>Detection of aneuploidy or amniocytes for molecular genetic analysis on fetal cells; determination of amniotic fluid A envy in Rh incompatibility (infrequent due to use of MCA-PSA); lamellar body count</td>
<td>Amniotic fluid leakage 1%–2%; spontaneous loss rate 0.7% if performed at 14 weeks or later</td>
<td>Reassurance or allows for genetic counseling, A envy guides interventions; lamellar body count indicates fetal lung maturity</td>
</tr>
<tr>
<td>Chorionic villus sampling</td>
<td>Detection of aneuploidy or molecular genetic analysis on fetal cells</td>
<td>If performed at an experienced center after 9 weeks, loss rate approaches that of mid trimester amniocentesis</td>
<td>Reassurance if normal, if abnormal, allows for genetic counseling</td>
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<tr>
<td>Cordocentesis: fetal hormones</td>
<td>Testing for congenital adrenal hyperplasia by CYP21 genotyping and assessment of antenatal steroid therapy by following marker 17-hydroxyprogesterone</td>
<td>&lt;2% Fetal loss related to procedure; 9.38% fetal bradycardia</td>
<td>Reduce risk of gender misassignment; possible reduction of gender confusion; may prevent need for corrective genital surgery for ambiguous genitalia</td>
</tr>
<tr>
<td>Cordocentesis: blood gases</td>
<td>Fetal acidemia</td>
<td>Same as above</td>
<td>Correlates with reduced development quotient; indicator for emergent delivery</td>
</tr>
<tr>
<td>Maternal site sampling for fetal products</td>
<td>Detection of fetal fibronectin for risk of premature labor; IL-6 and CRP in cervicovaginal fluid; use of α-microglobulin-1 test in cervicovaginal fluid</td>
<td>None</td>
<td>fFN: negative predictive value for labor, may reduce admissions; IL-6 and CRP proposed for chorioamnionitis; α-microglobulin-1 for detection of premature rupture of membranes</td>
</tr>
<tr>
<td>Meconium analysis; fetal or segmental maternal hair analysis</td>
<td>Detection of drugs of abuse and alcohol (fatty acid ethyl esters) exposures; environmental exposures</td>
<td>None</td>
<td>Allows for child protection, maternal rehabilitation; allows for removal of environmental risk and ongoing risk surveillance</td>
</tr>
<tr>
<td>Fetal urine analysis by percutaneous catheter</td>
<td>Assess need to decompress urinary obstruction: if Na &lt;100 mEq/L Cl &lt;90 mEq/L; osmolarity &lt;210 mosmol</td>
<td>Shunt can dislodge, obstruct, migrate</td>
<td>Avert renal damage; possibly promote lung growth</td>
</tr>
</tbody>
</table>

* Harrison et al. (1), Crombleholme et al. (2), Deprest et al. (3), Leviton et al. (6), Soothill et al. (30), Ghizzoni et al. (37), Sulcova et al. (38), Millar et al. (41), Forestier et al. (42), Daffos et al. (43), Rabinowitz et al. (44), Burc et al. (45), Bevis et al (46), Liley (47), Queenan et al. (48), Mari et al. (49), Siaan et al. (50), Gaer et al. (51), Gourley et al. (52), Verma et al. (53), Koren et al. (54), Chan et al. (55), Cemichiarri et al. (56), Loew et al. (61), Swamy et al. (62), Wei et al. (63), Ashwood et al. (64), Cousins et al. (65), Kingdom et al. (66), American College of Obstetricians and Gynecologists (68), Borgida et al. (69), Botto et al. (70), Brambati and Tuliu (71).

b Adapted with permission from Geaghan (94).
cart for easy transport to the maternal bedside (Fig. 2). The instrument must be optimized for small sample volume, ideally 25 μL or less to conserve fetal blood volume. Intravascular fetal blood transfusion by cordocentesis represents a significant therapeutic advance for red blood cell isoimmunization, or hemolytic disease of the newborn. Goals of limiting the duration of transfusion and reducing the risk of repeated procedures call for a brisk injection rate with maternally compatible transfused blood. Packed red cells are high hematocrit (ranging from 60% to over 80%), and represent significant transfused blood volumes (60–160 mL) (10,11). In one study of 12 fetuses expansion of fetoplacental blood volumes ranged from 36.34% to 106.37%, and the rate of increase from 2.41% to 16.47% per minute. Substantial expansion of the fetoplacental volume impacts cardiac dynamics; however, alterations are resolved within 2 h (12).

Following intrauterine transfusion, diagnostic blood testing reflects donor cells and plasma. Testing of posttransfusion samples can produce false-positive and false-negative test results, and cannot be relied upon for newborn screening or other definitive laboratory diagnostics. The role of the laboratory is to support cordocentesis procedures by offering technologies that minimize sample volumes and offer expedited turnaround times (most often, but not limited to, hemoglobin and hematocrit measurements).

Healthy Fetal Reference Intervals

Intrauterine biochemical assessments are evaluated with reference to gestational age and programmed development. Postnatal umbilical cord blood samples are routinely available but cannot be assumed to reflect fetal values, even at term. Colossal changes in the fetal physiology and anatomy occur during the transition from the in utero to the postnatal environment; for example, massive fluid shifts take place in the first hours of postnatal life, altering the distribution of body water (13). In the first postnatal week, physiologic contraction of the extracellular space accounts for an expected weight loss (attributable to water loss) of 5%–10% for term neonates (14) and 10%–20% for low birth weight/premature infants (15). Before birth, maternal and placental homeostatic mechanisms manage fetal water and electrolyte balance. After delivery, the neonate is subject to a more labile environment and must manage fluid and electrolyte changes that characteristically occur during the first days of life (16).

Values obtained from fetoscopic sampling from early mid- through late gestation have been used to establish reference intervals. These samples better reflect healthy fetal physiology than did earlier studies based on spontaneous or therapeutic abortuses. To establish fetal reference values for many analytes, the use of leftover blood from cordocenteses may be the only available option. Because cordocenteses carry substantial risks and are performed when fetal health is typically at risk or impaired, these blood samples do not ordinarily provide an ideal healthy reference population. With the use of strict exclusion criteria, however, robust data can be produced. Data collected from negative cordocenteses can provide reference values, ideally confirmed by physical examination at birth.

Small population sizes are associated with statistical uncertainty and wider confidence limits for the endpoints of each reference interval. Published consensus guidelines provide guidance on the use of percentiles to describe sample sizes (17).

The most extensive body of work that establishes healthy fetal reference intervals is found outside the clinical laboratory, in ultrasonographic measurement of fetal physical parameters of all types. Like laboratory measurements, these data are highly dependent on choice of method, are related to gestational age, and are critically important to inform the obstetrician about the health and development of the fetus. Recognized difficulties relative to accurate pregnancy dating and establishment of gestational age adds imprecision to

Fig. 2. Have instrument will travel.
Laboratory support cart for cordocentesis at the point of care.
data sets. It is recommended in the radiology literature to record measurements by gestational age in days (18). Recording of normative data by age in completed weeks allows for inclusion of fetuses that are up to 6 days older than the reference group age (as expressed in weeks), and contributes error. The recommendation to use age in days merits consideration in the laboratory community. The role of the laboratory is to provide the best available fetal reference data to support clinical decision-making in these specialized clinical settings.

Confirmation of Sample Integrity and Fetal Origin

Fetal laboratory diagnostics require that specimens can be definitively identified as fetal in origin (Table 3). Fetal red blood cells obtained by umbilical cordocentesis are distinguished from maternal cells by the Kleihauer–Betke test, or by flow cytometry that uses antibodies such as anti-hemoglobin F (HbF), and/or by differences between maternal and fetal mean corpuscular volume (MCV). The Kleihauer–Betke uses the different physical properties of HbF, the predominant hemoglobin in fetal red cells, and HbA, the predominant hemoglobin in adult cells, to determine source. A thin smear of maternal blood is treated with acid, rinsed, and counterstained. HbF in fetal cells is resistant to the acid elution treatment, so that fetal cells will stain bright pink with hemoglobin; in contrast, maternal cells with HbA-containing cells will appear as colorless “ghosts” following acid elution. The subjectivity of the test (microscopic categorization of cells as fetal or adult on the basis of staining characteristics) contributes to poor reproducibility. Flow cytometric analysis using a monoclonal anti-HbF antibody to quantify HbF-containing fetal cells is far superior in precision and objectivity in large multiyear multicenter trials, and in the aggregate experience of the national College of American Pathologists proficiency surveys (19).

However, flow cytometry preparation and analysis requires more time, and is therefore not suited for real-time guidance during the procedure. Confirmation of fetal identity also uses MCV measurements: maternal MCV is expected to be markedly lower than the developmentally large fetal MCV, without appreciable overlap in size ranges. A disease state, such as a macrocytic anemia, could certainly confound this determination, for example, by increasing maternal cell size, or MCV, into the fetal range. Likewise, increases in fetal hemo-
globin percentage in adults with disease states such as thalassemias are fairly common, and hereditary states of persistence of fetal hemoglobin in adult life are described, which could also (rarely) confound a Kleihauer–Betke test. For these reasons, a flow cytometric test offers a more reliable, reproducible, and superior metric, but is not yet widely employed and not available at the point of care. The assays currently available are primarily designed for another purpose: detection of fetal red cells in the maternal circulation, for prevention and treatment of maternal alloimmunization, or immune sensitization from exposure to paternal (foreign) antigens expressed by the fetus. In the College of American Pathologists HFB 2011-A, principal methods for identification of fetal hemoglobin across the US laboratories enrolled in proficiency testing programs included modified Kleihauer–Betke method (n = 854); immunoassays (n = 1730); and flow cytometric methods (n = 42), primarily anti-HgbF assays (20).

Amniotic fluid contamination is also a small risk in PUBS; in one series, contamination was reported in approximately 2.5% of umbilical blood samples (15/606) (7). Amniotic fluid has a lower pH (pH = 7.1) and other important chemical differences (higher lactate) compared with healthy fetal blood (21). The laboratory’s role is to confirm specimen integrity and fetal specimen identity for each fetal sampling by one of these technologies to assure quality and patient safety.

Biochemical Assessments of the Fetus

Knowledge of developmental biochemical as well as establishment of healthy fetal reference values is requisite for laboratory data interpretation. Certain analytes change appreciably as a function of gestational age and others do not. From a series of 171 fetuses that underwent PUBS between 18 and 40 weeks gestation, a subset of 72 healthy fetuses for biochemical analyses was identified after suspected pathologies were not confirmed. Physical examination and laboratory screening at birth confirmed these infants to be healthy. Testing for 18 biochemical analytes was performed, and the following trends noted: total protein and albumin plasma concentrations increase with advancing age, but remain lower than those in adults (22); this finding was replicated in several studies (23, 24). Creatine kinase increases with gestational age, then falls at term to neonatal concentrations. Alkaline phosphatase peaks at 21–25 weeks at concentrations higher than those in adults, and then falls to concentrations lower than in adults; this pattern has also been confirmed in several studies (23, 24). Concentrations of sodium, potassium, urea nitrogen, creatinine, lactate dehydrogenase, uric acid, and magnesium are not substantially different in fetuses than in term newborns. Cholesterol and triglyceride concentrations are much lower than adult concentrations. Fetal total bilirubin concentrations are lower than in neonates, but higher than in adults. Also notable are phosphorus and γ-glutamyltransferase enzymatic activity levels, which are higher in fetuses compared with both newborns and adults. In the fetus, activity levels of enzymes such as aspartate aminotransferase and alanine aminotransferase appear to be independent of maternal levels, and far lower than those of other adults (24–27). Values from multiple studies are consistent, but differences exist owing to differences in instrumentation and small sample size.

The acquisition of passive immunity from maternal source immunoglobulin is evident in the increase in fetal IgG from week 20 to 34, from a mean of 256 to a mean of 566 mg/dL. (P < 0.001). Fetal immunoglobulin A and M, representing solely fetal production, also appeared to increase with gestational age, but the values did not reach statistical significance (27). The majority of the maternal IgG is transferred to the fetus during the last 4 weeks of pregnancy, rendering premature infants relatively deficient in maternal antibodies (28). These trends are important manifestations of the ontogeny of the fetal immune response. Could fetal blood biochemical analysis be helpful for timing of delivery? Fetal cord sampling for oxygenation and acid–base balance demonstrates fetal pH to be slightly higher than maternal pH, but close to 7.40; fetal PO2 ranges in the 60s and 70s for the majority, and fetal percentage saturations from the high 70s to the high 80s. Human fetal umbilical vein pH and PO2 are higher, and Pco2 much lower than those observed at delivery (27). In growth-retarded human fetuses, cord sampling for blood gases demonstrated that for fetuses in whom clinical assessment (independent and blinded to laboratory values) warranted immediate delivery, pH was lower (P < 0.001), and Pco2 higher (P < 0.002) compared with fetuses with more favorable clinical assessments, indicating that the pregnancies should be allowed to continue (29). Fetal lactate concentration was inversely correlated with pH (P < 0.0009). Another study showed an association between chronic fetal acidemia and subsequent impaired neurodevelopment, as measured in 36 fetuses who had cordocenteses for growth retardation. Those who had acidemia as fetuses had significantly lower developmental quotients (30). These data suggested that fetal biochemical analyses could be helpful for clinical decision-making regarding timing of delivery. Fetal blood sampling for pH and lactate has been employed when further information about the fetal status is desired, in the presence of an abnormal or non reassuring fetal heart rate pattern. One review of the literature found a statistically significant higher sample yield rate for lactate compared with pH sampling, but no reported difference in correlation.
with neonatal or maternal outcomes (31). Fetal scalp pH and lactate sampling are technologies that were introduced into practice, as was fetal electric monitoring by use of cardiac tocography (32), before a clear evidence base in terms of neonatal/child/maternal outcome was established, and benefit remains unproven.

Fetal laboratory measurements may potentially offer prognostic information regarding postnatal organ function. A prospective noninterventional study measured cystatin and \( \beta_2 \)-microglobulins (low molecular weight proteins that are markers of kidney function) for the prediction of renal function impairment in excess serum from 129 cordocenteses in 84 fetuses. Maternal and fetal serum concentrations did not correlate for either cystatin or \( \beta_2 \)-microglobulin, but correlated closely for serum creatinine. For the prediction of renal dysfunction, creatinine from the mother confounds fetal assessment. Overall, mean plus 1 SD for cystatin C was 1.66 (0.202) mg/L (upper limit of reference) founds fetal assessment. Overall, mean plus 1 SD for cystatin C was 1.66 (0.202) mg/L (upper limit of reference interval at mean plus 1.96 SD = 2.06 mg/L), independent of gestational age. \( \beta_2 \)-microglobulin, in contrast, decreased with gestational age; the upper limit was established as a dynamic range determined to be 7.19–0.052 mg/L, multiplied by gestational age in weeks. Cystatin had a higher specificity for detection of renal dysfunction (91.8%) compared to \( \beta_2 \)-microglobulin (85.5%), whereas \( \beta_2 \)-microglobulin had a higher sensitivity than cystatin (90.0% vs 63.6%), and diagnostic efficiency for the 2 tests was equivalent. Cystatin is produced by nucleated cells; \( \beta_2 \)-microglobulin is produced by lymphocytes and more likely to vary with infections and other factors (33). However, a large systematic review and metaanalysis concluded an overall poor predictive accuracy for fetal urinalysis (34). Instead, percutaneous fetal cystoscopy (direct visualization) may determine those urinary tract lesions that are appropriate for in utero therapy: a recent systematic review found that fetal cystoscopy revised the ultrasound pathologic diagnosis in 25%–36.4% of fetuses (35). This evidence was limited to 2 series. At present, measurement of amniotic fluid volume and the appearance of the renal (parenchymal) cortex appear to be the most predictive features of poor postnatal renal function (36). However, this disappointing review of urinalysis is likely attributable to confounding factors, including: lack of use of gestational age specific reference intervals in studies, different assay methodologies, and lack of definitive cutoff values.

Prenatal endocrine analysis is another area of fetal diagnostic impact. Congenital adrenal hyperplasias are a group of autosomal recessive endocrine disorders attributable to enzyme deficiency in steroid synthesis. The most common etiology is \( 21 \)-hydroxylase (21-OH) deficiency. The goal of prenatal diagnosis and treatment and of 21-OH deficiency is prevention of prenatal virilization in affected female fetuses, and avoidance of consequences such as risk of gender misassignment, gender confusion, and indications for possible corrective genital surgery (37). Early diagnosis (before the 15th gestational week) is desirable. Genotyping for the responsible \( CYP21 \) (cytochrome \( P450 \) family 21, subfamily \( A \), polypeptide 2)\(^1\) gene can be performed from chorionic villi sampled at 10–11 weeks gestation, although technical factors can lead to diagnostic mishaps (37). Amniotic \( 17 \)-hydroxyprogesterone is the diagnostic biochemical marker for 21-hydroxylase deficiency, is unaffected by sex, and variation with gestational age is not significant (38). Guidance for fetal treatment of congenital adrenal hyperplasia is therefore possible by monitoring \( 17 \)-hydroxyprogesterone during antenatal dexamethasone treatment. Although outcome studies of patients after antenatal exposure to dexamethasone have shown no significant adverse effects, confirmation of the safety of this treatment (e.g., on brain development) awaits longer-term randomized controlled studies of treated vs untreated pregnancies (38).

For other endocrine disorders, fetal studies are not available, and only data from preterm births are available. For example, luteinizing and follicle-stimulating hormone concentrations have been reported for premature infants at 24–29 weeks (39).

Ideally, collaboration among laboratories involved in fetal sampling at maternal fetal medicine centers could lead to the creation of multiinstitutional data sets (for example, by investigations on excess samples, when available) to expand the limited literature in fetal biochemistry, and better characterize health and disease states in the antenatal setting.

**Hematologic Assessments of the Fetus**

Fetal hematologic measurements inform our understanding of developmental hematopoiesis and help guide transfusion therapy (Table 4) (40). Fetal red cell count, hemoglobin, and hematocrit sequentially rise over the course of gestational weeks 15 through term. Simultaneously, the red cell MCV gradually and progressively falls to term. Total white blood cell (WBC) count also rises from the late second trimester to term. Total WBC count must be manually corrected in the presence of fetal nucleated red blood cells. Without correction, total WBC count overestimation occurs because of impedance count interference from the similar-sized nucleated red blood cells in the WBC channel. Quantitative WBC counts in the second-trimester fetus are a fraction of the numbers observed in the term neonate. Platelet counts, on the other hand, reach levels considered to be adequate for older children and adults during the
second and third trimesters, and continue to rise in number to term (41, 42).

Prenatal diagnosis and management of hereditary and acquired immune bleeding disorders by fetal blood sampling has been described in 103 cases, allowing for in utero transfusions of coagulation factor concentrates and platelets; assessment of success of certain maternal therapies for fetal conditions; and effective planning for minimizing trauma by the caesarean mode of birth (43).

This area of fetal hematology and coagulation testing would also benefit from multiinstitutional consortiums; collective experience could expand the current limited fund of knowledge on fetal hematologic ontogeny and also serve to better characterize fetal health and disease states.

### Alternative Sample Matrices for Fetal Health Assessment

Several alternative matrices are clinically useful for fetal health assessment. Fetal urine production increases briskly with gestational age, from 5 mL/h at 20 weeks gestation to 51 mL/h at 40 weeks gestation (44). Fetal urine production is the primary component of amniotic fluid. Amniotic fluid, obtained by invasive amniocentesis, is one of the best-studied matrices for fetal analysis. The composition varies throughout gestation: in the first trimester, it is essentially a plasma ultrafiltrate. Later, in the second and third trimesters, the developing renal, urinary, gastrointestinal, and pulmonary organ systems contribute their secretory products to amniotic fluid to yield a more complex matrix (45).

The first widespread application of amniotic fluid analysis was for prenatal assessment for Rh-group immunized pregnancies. In these pregnancies, the fetus is Rh-positive and mother Rh-negative, causing an immune-mediated maternal destruction of fetal red cells leading to severe anemia and even intrauterine death. The field advanced in 1953 when Bevis et al. demonstrated that amniotic bilirubin pigment concentrations correlate with clinical hemolytic disease of the newborn (46). The Liley system for prediction of fetal condition was based on amniotic fluid absorbance, spectrophotometrically measured at wavelength 450 nm ($A_{450}$). This system, introduced in the 1960s, was clinically useful in categorization of pregnancies 27 weeks to term into mild/absent and severe disease, with a mid-zone requiring repeat testing (47). However, the inability of this system to be extrapolated to earlier gestational ages was a limitation better addressed by the generation of the Queenan system. This dataset, also based on amniotic fluid $A_{450}$, spanned 14 – 40 weeks gestation, and offered a new management protocol based on 4 zones of risk (unaffected, indeterminate, affected, and intrauterine death zone). Excellent clinical correlation and outcomes were demonstrated on the basis of a dataset of 789 pregnancies (48).

In 2000, the practice of fetal medicine changed with the demonstration that noninvasive Doppler ultrasound of the fetal middle cerebral artery peak systolic velocity (MCA-PSV) could be used to better assess and guide treatment of hemolytic disease of the newborn, as first reported by Mari et al. (49). The study included 111 fetuses at risk for hemolytic disease of the newborn due to red cell isoinmunization, and 265 healthy fetuses. A nomogram was created using a reference interval for gesta-

### Table 4. Hematologic parameters in healthy fetuses by gestational age,a,b

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Sample no.</th>
<th>Hemoglobin, g/dL</th>
<th>Red blood cells, $x 10^6$/L</th>
<th>Hematocrit, %</th>
<th>MCV, fl</th>
<th>Total WBC count, $x 10^3$/L</th>
<th>Corrected WBC count, $x 10^3$/L</th>
<th>Platelets, $x 10^3$/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>15c</td>
<td>6</td>
<td>10.9 (0.7)</td>
<td>2.43 (0.26)</td>
<td>34.6 (3.6)</td>
<td>143 (8)</td>
<td>1.6 (0.7)</td>
<td>—</td>
<td>190 (31)</td>
</tr>
<tr>
<td>16c</td>
<td>5</td>
<td>12.5 (0.8)</td>
<td>2.68 (0.21)</td>
<td>38.1 (0.21)</td>
<td>143 (12)</td>
<td>2.4 (1.7)</td>
<td>—</td>
<td>208 (57)</td>
</tr>
<tr>
<td>17c</td>
<td>16</td>
<td>12.4 (0.9)</td>
<td>2.74 (0.23)</td>
<td>37.4 (0.28)</td>
<td>137 (8)</td>
<td>2.0 (0.8)</td>
<td>—</td>
<td>202 (25)</td>
</tr>
<tr>
<td>18–21d</td>
<td>760</td>
<td>11.69 (1.27)</td>
<td>2.85 (0.36)</td>
<td>37.3 (4.32)</td>
<td>131.1 (11.0)</td>
<td>4.68 (2.96)</td>
<td>2.57 (0.42)</td>
<td>234 (57)</td>
</tr>
<tr>
<td>22–25d</td>
<td>1200</td>
<td>12.20 (1.6)</td>
<td>3.09 (0.34)</td>
<td>38.59 (3.94)</td>
<td>125.1 (7.8)</td>
<td>4.72 (2.62)</td>
<td>3.73 (2.17)</td>
<td>247 (59)</td>
</tr>
<tr>
<td>26–29d</td>
<td>460</td>
<td>12.91 (1.38)</td>
<td>3.46 (0.41)</td>
<td>40.88 (4.4)</td>
<td>118.5 (8.0)</td>
<td>5.16 (2.53)</td>
<td>4.08 (0.84)</td>
<td>242 (69)</td>
</tr>
<tr>
<td>&gt;30d</td>
<td>440</td>
<td>13.64 (2.21)</td>
<td>3.82 (0.64)</td>
<td>43.55 (7.2)</td>
<td>114.4 (9.3)</td>
<td>7.71 (4.99)</td>
<td>6.4 (2.99)</td>
<td>232 (87)</td>
</tr>
</tbody>
</table>

* Hematologic measurements by a Coulter S-plus II instrument on 2860 fetal blood samplings for prenatal diagnostic purposes. Data expressed as mean (SD). Total WBC count includes NRBCs. Corrected WBC count includes WBCs only, after subtracting NRBCs, based on a 100-cell count manual differential.
* Adapted with permission from Geaghan (94).
* Data from Millar et al. (41).
* Data from Forestier et al. (42).

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**Clinical Chemistry** 58:2 (2012) 9
tional age–specific hemoglobin concentration developed from healthy fetuses at 18–40 weeks gestation, and gestational age–specific reference values developed for the MCA-PSV. It was demonstrated that with the use of this nomogram and measurement of MCA-PSV, 70% of invasive cordocenteses could have been avoided. In these cases, hemoglobin sampling by cordocentesis revealed that the fetuses were only mildly or not at all anemic. By using the decision limit of a multiple of the mean of 1.5 or greater for MCA-PSV, all significant anemias would have been identified and none missed, with a false-positive rate of 12%. Additionally, in the remaining 30% of affected fetuses, the fetuses were already hydramic (indicating advanced disease) in 40% of cases, but could have been identified earlier without risk of invasive procedure, by serial Doppler MCA assessments. Doppler MCA velocimetry offers utility in noninvasive diagnosis and management of other types of fetal anemia, and is now pivotal in assessment of intrauterine growth retardation.

The study of enzymes in amniotic fluid between 14 and 35 weeks gestation demonstrates that timely and reliable amniotic enzyme quantification is possible on a conventional analyzer, with minor adaptation. In cases of sonographically diagnosed bowel disorders, assays of amylase, γ-glutamyl transferase, 5′-nucleotidase, and total alkaline phosphatase were found to be of diagnostic value as confirmatory tests. For example, γ-glutamyl transferase and 5′-nucleotidase have high activities in bile, and are therefore low (<5th percentile) activities in biliary atresia due to restriction of bile flow. Increases in all 5 enzymes are associated with gastroschisis, a congenital defect in the anterior abdominal wall through which the abdominal organs freely protrude. In these cases, enzyme activities are usually at >95th percentile, due to the open communication between amniotic fluid and the bowel in that disorder (45).

Glycosaminoglycan accumulation from fetal urine has also been analyzed in amniotic fluid. Chondroitin sulfate, the dominant human glycosaminoglycan, together with hyaluronic acid comprise the majority found by amniotic fluid analysis; heparin sulfate and dermatan sulfate are present in small amounts and undetectable in healthy pregnancies, respectively. Relative changes in the proportions of these components have been noted in several congenital metabolic disorders, and may have diagnostic value (50).

A matrix entirely unique to the fetus is meconium, the fetal waste product accumulated over the second and third trimesters of gestation. In term infants, 99% pass their first formed stool by 48 h. Meconium is completely evacuated within the first 125 h after birth, and for analytic purposes, collection is recommended within the first 72 hours of postnatal life (thereafter the matrix is a transitional one, from meconium to feces). In preterm, extremely low birth weight infants, the evacuation of meconium is comparatively delayed: median age at first stool is 3 days, with 90% of infants evacuating meconium by 12 postnatal days (50–53). Meconium analysis is therefore a retrospective evaluation of fetal exposures in utero, but nonetheless a highly valuable tool for assessment. Specific utilities include testing for environmental toxin exposures and maternal drugs of abuse. According to current understanding, once meconium is deposited, this matrix represents a stable record of antenatal exposures.

Exposures to alcohol, drugs of abuse, and smoking present variable degrees of risk to the fetus, mother, and family. The use of segmental hair analysis is helpful in maternal–fetal pharmacology and toxicology, facilitating identification of chronological patterns of drug and alcohol abuse in pregnant women. This matrix is unique, because detection of patterns of illicit drugs of abuse and prenatal alcohol use over time is possible, including confirmation of abstinence or changes in drug abuse for child protection (54). Fatty acid ethyl esters are a marker of prenatal alcohol exposure available from segmental hair analysis, and nicotine concentrations are available as a marker for maternal smoking (55). Maternal hair analysis is also recommended for monitoring prenatal exposure to methylmercury. Methylmercury poses an environmental risk to which the fetus is exquisitely sensitive owing to neurotoxic effects on the developing central nervous system. As a stable, historical record of concentrations of transportable species in plasma, hair is analyzed as a surrogate for fetal tissue (brain) concentrations (56). Noninvasive collection, easy and inexpensive transport and storage, and minimal issues of sample integrity make hair an ideal matrix for analysis.

The spectrum of bioanalytic matrices and methods to monitor in utero drug exposures also includes vernix caseosa (thick lipid and cellular fetal covering), oral fluid, amniotic fluid, urine, sweat, blood, hair, nails, and cord tissue (57). Although these measurements actually take place at or after birth, they are retrospective assessments of the exposures and environment of fetal life. The clinical laboratory’s role is to expand the repertoire of available matrices for testing in the future, or to make such options available on a referral basis, as clinically appropriate.

**Fetal Secretory Products Accessible by Maternal Sampling**

The identification or quantification of fetal-specific secretory products (in relation to gestational age) in accessible maternal sites can offer valuable antenatal diagnostics and aid decision-making for preterm labor and delivery. Maternal serum α-fetoprotein (AFP) screening is offered between 14 and 22 weeks gestation,
as part of evolving algorithms and often in combination with additional tests, as a marker for neural tube defects and a variety of common congenital abnormalities. AFP is a fetal glycoprotein that is synthesized in the yolk sac early in gestation, and is later formed in the fetal liver and gastrointestinal tract. Though AFP constitutes the major serum protein in the fetus, its function is unknown. AFP circulates in extraordinarily high concentrations in fetal serum, passing into the fetal urine, which is the major contributor to amniotic fluid. The concentration of AFP in fetal serum and in amniotic fluid peaks at 13 weeks and decreases thereafter. After 12-weeks gestational age, this protein is found in increasing concentrations in maternal serum, because it diffuses across fetal membranes and is also transported by diffusion into the maternal placental circulation. Measurement of maternal serum AFP is expressed as a multiple of the mean of a healthy population. This reporting mechanism is used to normalize AFP values to a statistical distribution, and facilitate comparison of screening program results across different populations and laboratories. These maternal measurements of a ubiquitous fetal protein form the basis of widely adopted prenatal screening programs, and combined with 2 or 3 additional tests (known as a “triple” or “quad” screen, the details of which are outside the scope of this review) can help identify a wide variety of fetal conditions.

Fetal-specific fibronectin (fFN) is an extracellular glycoprotein uniquely produced by fetal placental tissue, also known as “trophoblast glue” for its role in securing placental trophoblasts to maternal decidua. fFN detection in cervicovaginal secretions can be used as a prognosticator for preterm labor. fFN is measured on samples free of blood and amniotic fluid by enzyme-linked immunosassay; a positive value exceeds 50 μg/L. In a randomized trial, a negative fFN in women presenting with threatened preterm labor was linked to fewer hospital admissions and decreased length of hospital stay. A robust negative predictive value of 98% for delivery within 1 week of an assay performed at 22–34 weeks is useful; positive predictive values are much lower. The poor positive predictive value has discouraged use of this test; however, a negative predictive value can potentially be leveraged in an appropriate clinical algorithm. Inflammatory cytokine interleukin-6 (IL-6) in cervicovaginal fluid, and both IL-6 and C-reactive protein (CRP) in amniotic fluid are strongly associated with spontaneous preterm birth in asymptomatic women. This strong association is not found for IL-6 and CRP in maternal plasma, suggesting that inflammation occurring at the maternal–fetal interface, rather than systemic inflammation, could be the key factor in spontaneous preterm births. Clinical application awaits development of standardized assays for detection of increased IL-6 and CRP in cervicovaginal fluid to assess the utility in obstetric care.

The quantification of lamellar body counts from third trimester amniotic fluid provides evidence of lung maturity, and is predictive for respiratory status postdelivery. Measurement of maternal serum α1-microglobulin-1, is present at much higher concentrations in amniotic fluid compared with cervicovaginal fluid. When high concentrations of this protein are detected in cervicovaginal fluid, a diagnosis of rupture of fetal membranes is made with a high degree of accuracy; a sensitivity of 98.0%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 99.1% have been reported.

The role of laboratories that support obstetric patients is to offer rapid turnaround times on these maternal samples of fetal products (lamellar body counts, placental α-microglobulin-1, and specific markers of preterm labor as the evidence base develops) for expedited clinical decision-making.

Fetal Laboratory Testing Contributes to Understanding of Disease

Fetal studies can elucidate, challenge, and revise our understanding of the natural history of fetal diseases in important ways. Twin–twin transfusion syndrome, manifested in 4%–25% of monochorionic multiple pregnancies, is responsible for 17% of twin mortality. The syndrome is posited to be due to vascular anastomoses (connections) between twin circulations, particularly of placental anastomoses (connections) between twin circulations.
and inadequate compensatory vascular communications. Unbalanced unidirectional flow becomes evident early in the second trimester, with discordant fetal growth and unequal amniotic sac fluid volumes. The “donor” twin is growth retarded and oliguric, with oligohydramnios (insufficient amniotic fluid volume); the “recipient” twin is larger and polyuric, with polyhydramnios (excess amniotic fluid volume). Studies of fetal iron metabolism in monochorionic twin pregnancies with twin–twin transfusion syndrome have demonstrated higher ferritin concentrations in recipient twins than in donor twins in utero; however, these concentrations, as well as stable postnatal liver iron, appear to be comparable to twins without this syndrome. These data fail to support a theory of iron overload in recipient twins, and iron depletion in donor twins. In fact, recipient ferritin concentrations were far below those associated with iron overload (which are >1000 μg/L), and donor ferritin concentrations were within reference intervals expected for fetuses from singleton pregnancies, sampled for intrauterine growth retardation (67). Such studies challenge our theories and augment our knowledge of fetal health and disease in the high-risk group of monochorionic twins. Again, use of discarded or leftover fetal blood samples from maternal–fetal medicine centers is a largely untapped resource for potential investigation of fetal parameters.

Prenatal Testing for Aneuploidy and Future Directions in Fetal Laboratory Diagnostic Evaluation

Invasive prenatal testing for fetal aneuploidy includes conventional amniocentesis offered at 15–20 weeks gestation and chorionic villus sampling (CVS), offered after 9 weeks completed gestation, respectively. Cyto-genetic accuracy is >99% on these samples. The procedure–related fetal loss rate for CVS appears to approach that of midtrimester amniocentesis, about 1 in 300–500, or lower depending on the center (68). For amniocentesis the rate of cell culture failure is 0.1% of samples. Complications such as amniotic fluid leakage and vaginal spotting occur in 1%–2% of all amniocenteses; chorioamnionitis occurs in <1 in 1000 cases (69). For CVS, complications include vaginal spotting or bleeding in up to 32.2% of patients after transcervical CVS, less if the procedure is performed transabdominally. Initial reports of limb reduction anomalies were linked to procedures before 9 weeks of age (70). The overall complication rate, including amniotic fluid leakage infection and culture failure, is <0.5% (71).

Most recently, the American College of Obstetricians and Gynecologists Committee on Genetics recommended that targeted (rather than genome-wide) array comparative genomic hybridization be offered for fetal abnormal sonographic anatomic findings and a normal karyotype, or for fetal demise cases without karyotype. Despite improved resolution over conventional karyotype for detecting chromosomal abnormalities <3 Mb, limitations of the targeted array include inability to detect balanced chromosomal rearrangements, uncertain significance of copy number variations, and higher costs, and give caution to widespread screening application (72).

The discovery of fetal cell-free nucleic acids in plasma of expectant mothers has led to the development of noninvasive technologies for antenatal diagnosis of fetal aneuploidy. Fetal DNA is detectable as early as day 18 after embryo transfer in cases of assisted reproduction. Advantages of very early detection, without the possibility of procedure-related loss of an unaffected fetus, hold great promise. The first report of fetal aneuploidy detection by high-throughput shotgun sequencing of cell-free DNA, and mapping of short sequences to the chromosome of origin, was reported in 2008 (73). Small changes in numerical representation of chromosomes, detected by counting millions of DNA sequences, are indicative of an aneuploid fetus. The overrepresentation of chromosomes 13, 18, and 21 in trisomies and underrepresentation of chromosome X in male pregnancies was demonstrated, and findings have been independently reproduced (74, 75). Progress on several challenging technical areas follows.

First, the portion of the DNA sample derived from the fetus is comparatively small. The high background of maternal DNA limits assay sensitivity. The magnitude of the fetal DNA fraction varies between pregnancies and throughout gestation (76). Many investigators have reported <10% fetal DNA from sequencing studies (77). Using high-throughput paired-end sequencing, size distributions of maternal and fetal cell-free DNA have confirmed that cell-free DNA has a peak size of 160–180 bp, and originates primarily from apoptotic cells. Fetal DNA is shorter (usually <500 bp) than maternal DNA (78, 79) and techniques to enrich for shorter (<150 bp) DNA to increase sensitivity are a focus of study. The sensitivity for fetal aneuploidy detection is dependent on the original amount of fetal DNA, the relative enrichment by size selection techniques, and the residual number of molecules for analysis after size selection. Enrichment techniques can reduce the number of molecules available to be counted (80). Techniques to increase assay sensitivity for fetal genotyping or detection of fetal point mutations by fetal DNA enrichment also include use of smaller PCR amplicons (81), microchips (77), and techniques (74, 75, 82, 83) to extract lower molecular weight DNA fractions. These technologies offer a radically shortened turnaround time for aneuploidy detection. Microfluidic digital PCR, for example, uses uncultured amniocytes and
On the Frontier of Maternal–Fetal Medicine

Chiu et al. compared massively parallel sequencing of maternal plasma DNA against full karyotyping on 753 pregnancies at high risk for trisomy 21, using prospectively collected and archived maternal samples (85). No prior validation of the diagnostic accuracy and feasibility analysis of multiplexed maternal plasma DNA sequencing for trisomy 21 detection had been conducted on a large scale. Two protocols with different sample throughputs were tested: a 2-plex and an 8-plex sequencing protocol. The 2-plex protocol demonstrated superior results, available for 314 pregnancies: 100% sensitivity and 97.9% specificity, and a positive predictive value of 96.6% and negative predictive value of 100%. The efficacy of the noninvasive sequencing test suggests that a reduction in the number of high-risk pregnancies requiring an amniocentesis or a CVS, both of which carry procedure-associated risk, is possible (85). Investigators have demonstrated that targeted sequencing of genomic regions using enrichment kits allows for increased coverage of fetus-specific alleles within targeted regions; this may increase throughput and reduce costs compared with nonselectively sequencing the genome. Protocols that combine bioinformatics and sequencing can noninvasively survey the entire genome of a fetus for mutations and genetic loci (86).

The minimum amount of requisite fetal DNA fraction (i.e., the number of DNA molecules large enough to be statistically meaningful) and depth of sequencing for different diagnostic targets is in the process of definition through additional clinical trials.

In the immunohematology arena, an important limitation for fetal blood-type genotyping was an immunologic phenomenon: fetal–maternal ABO or Rh incompatibility can limit the lifespan and survival of fetal red cells in the maternal circulation (87). Free fetal DNA analysis allows for such genotyping despite immunological destruction of fetal cells; a novel multiplex assay to detect the fetal Rh blood group, D-antigen gene (RHD) loci is one example. This RHD genotyping assay includes: exons 4, 5, 7, and 10; the RHDp pseudogene of the RHD gene; the sex-determining region, or Y chromosome–specific assay; and a generic PCR amplification control. Plasma samples from 150 randomly selected pregnant women were assayed for fetal RHD genotype using the MassARRAY system. The fetal RHD status of 148 of 150 samples (98.7%) was correctly classified; 86 (57.3%) and 62 (41.3%) were positive and negative, respectively. Routine fetal RHD genotyping using a multiplex assay on cell-free fetal DNA collected from maternal plasma holds several advantages, as described in editorial commentary: neither mother nor fetus is exposed to the risks associated with invasive procedures such as amniocentesis or chorionic villus sampling, including the risk of immunological sensitization associated with invasive procedures. Furthermore, routine testing of nonimmunized RhD-negative pregnant women may be justified to avoid unnecessary cost and use of RhD immune globulin. When compared with real-time PCR, the multiplexing of the MassARRAY system allows for many loci to be analyzed simultaneously, so that only a single reaction is required. Lastly, RHD variants that yield negative antigen presentation but do not represent an entire gene deletion (e.g., the ψ pseudogene or various exon conversions) can be correctly typed from a single small sample of cell-free fetal DNA in a single reaction (88). Comparison of this assay with current serologic testing in broad-based clinical trials will more fully characterize the clinical utility.

Ethical issues raised by noninvasive prenatal testing include nonclinical applications of the technology for early determination of fetal sex and paternity testing as well as performing such testing without adequate informed consent (89, 90). The potential for prenatal screening without procedural risks may allow for widespread accessibility and usage. Commercial tests for aneuploidy detection have recently been approved in the US; by early 2012, 2 are likely to be available (Dr. Steven Quake, personal communication, December 6, 2011). Concerns include additional cost for each pregnancy, and to the healthcare system(s) if the risk reduction of noninvasive prenatal diagnosis leads to widespread demand. Without adequate genetic counseling resources, the test results may not lead to a well-informed choice. In both India and China, unbalanced sex ratios have led to prohibition of prenatal sex selection for social reasons (91, 92). Internet advertisement and regulation of consumer access are also areas of controversy. Fetal interventions in the future are also likely to include reconstruction of congenital fetal defects in utero from multipotent mesenchymal stem cells obtained by amniocentesis and use of biologic matrices, an active and promising area of investigation in tissue engineering (82). The clinical laboratory’s role will be one of support and guidance, and will evolve along with this therapeutic frontier.

SUMMARY

As the field of fetal surgery advances, new techniques will allow additional congenital defects to be treated, and an expanded portfolio of minimally invasive technologies will be developed. Laboratory support of these surgical interventions, when required, mandates: miniaturized diagnostic blood methodologies, the ability to provide blood counts in an expedited fashion to assess for intrauterine transfusion requirements, and appropriate technologies for biochemical assessment and continuous monitoring of the fetus (94). The laboratory community can contribute to improved fetal health by studies of developmental changes in fetal bio-

Clinical Chemistry 58:2 (2012) 13
chemical and hematologic parameters in health and disease on discard samples, and by analysis of our institutional and collective outcome-based monitoring experience. Although opportunities for noninvasive antenatal diagnosis and fetal therapeutics are limited only by the imagination, the application of such technologies for nonmedicinal purposes (sex selection) underscores the need to develop ethical approaches for implementation, in concert with the technologies.

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