The National Academy of Clinical Biochemistry

Presents

LABORATORY MEDICINE PRACTICE GUIDELINES

MATERNAL-FETAL RISK ASSESSMENT AND REFERENCE VALUES IN PREGNANCY

MATERNAL-FETAL RISK ASSESSMENT AND REFERENCE VALUES IN PREGNANCY

Printed by: **Durik Advertising, Inc.**
123 SE Parkway Ct, Suite 120
Franklin, TN 37064
phone: 615-794-8993
wdurik@durikadvertising.com

GUIDELINES COMMITTEE

EDITOR

John E. Sherwin, PhD, DABCC, FACB
Chief, Genetic Disease Laboratory, Department of Health Services of California, Richmond, CA

COMMITTEE

Gillian Lockitch, MD, MBChB, FRCPC
Professor, Pathology & Laboratory Medicine, University of British Columbia; Director of Laboratories, and Head, Department of Pathology and Laboratory Medicine, Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada

Philip Rosenthal, MD, FAAP, FACH, FACG
Professor of Pediatrics and Surgery, University of California, San Francisco, CA

Stephanie Rhone, MD, RDMS, FRCSC
Clinical Assistant Professor, Department of Obstetrics and Gynecology and Centre for Healthcare Innovation and Improvement, University of British Columbia and the Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada

Laura A. Magee, MD
Internist (Medical Disorders of Pregnancy), Children's and Women's Health Centre of BC, Vancouver, BC, Canada

Edward R. Ashwood, MD, FACB
Professor of Pathology, University of Utah, Director of Laboratories and Chief Medical Officer, ARUP Laboratories, Inc., Salt Lake City, UT

Barbara M. Goldsmith, PhD, FACB
Director, Laboratory Services, Caritas St. Elizabeth's Medical Center, Boston, MA

Carol R. Lee, MS (ret.)
Beckman Coulter, Inc., Chaska, MN

Sharon Geaghan, MD
Clinical Laboratory, Lucile Packard Children's Hospital, Palo Alto, CA

David Millington, PhD
Duke University Medical Center, Pediatrics, Medical Genetics, Research Triangle Park, NC

Michael Bennett, PhD, FACB
Department of Pathology, Children's Medical Center of Dallas, Dallas, TX

OTHER CONTRIBUTORS

Peter von Dadelszen, PhD, MBChB, FRCSC, MRCOG
Perinatologist, Children's and Women's Health Centre of BC, Vancouver, BC, Canada

Bob Currier, PhD; George Helmer, PhD; and Fred Lorey, PhD
Genetic Disease Laboratory, State of California, Richmond, CA

This publication is produced with the assistance of the following NACB Committees:

NACB PUBLICATIONS COMMITTEE

Kiang-Teck Yeo, PhD, DABCC, FACB (Chair)
Dartmouth-Hitchcock Medical Center and Dartmouth Medical School, Lebanon, NH

Charlie Hawker, PhD, DABCC, FACB
ARUP Laboratories, Inc., Salt Lake City, UT

Stanley Lo, PhD, DABCC, FACB
Children's Hospital of Wisconsin, Wauwatosa, WI

James Ritchie, PhD, FACB
Emory University Hospital, Atlanta, GA

Sayed Sadrzadeh, PhD, FACB
University of Washington School of Medicine, Seattle, WA

NACB EDUCATION AND SCIENTIFIC AFFAIRS COMMITTEE

Catherine Hammett-Stabler, PhD, DABCC, FACB (Chair)
University of North Carolina, Chapel Hill, NC

Michael Bennett, PhD, FACB
Children's Hospital of Philadelphia, Philadelphia, PA

D. Robert Dufour, MD, FACB
10316 Gainsborough Rd., Potomac, MD

Shirley Welch, PhD, DABCC, FACB
Kaiser Permanente NW Regional Laboratory, Portland, OR

TABLE OF CONTENTS

Preamble to the Guidelines

Children aren't just small adults….

The 1997 version of the NACB guidelines was devoted to the special issues surrounding the newborn and generated about 75 recommendations that spanned a host of topics, including collection of specimens in the newborn, reference intervals, and blood gas analysis. There were suggestions for stat and optimal turn-around times, the use of appropriate and specific analytes in this population, bilirubin and liver function tests, TM and pharmacokinetics as applicable to the newborn, and a discussion on detection of illicit drugs. Many of these recommendations still stand today.

When this version was first posted in 2003, the committee believed it was important to include and address the expanded role of the clinical laboratory in the assessment of fetal and maternal risk. This includes guidelines for laboratory testing and risk assessment that truly encompass the total time period from the confirmation of the new pregnancy through a healthy delivery for both mother and child, and the early detection of hidden health problems through newborn screening.

Many new point-of-care testing technologies for the assessment of the newborn have emerged. It is therefore worthwhile to include in these guidelines the enhanced role of point-of-care testing within neonatal laboratory medicine.

The emergence of new technologies and markers often occurs at a much higher velocity that can be captured, validated, put into accepted practice, and reviewed for inclusion in these guidelines. For example, in the prenatal screening area, there is considerable discussion of both second trimester markers such as ITA and Adam 12, but they are not yet in routine practice, in contrast to Inhibin A. The issue of first-trimester screening and nuchal translucency is still being widely discussed. One potential reference is the recent paper by David Wright and Ian Bradbury (BJOG 2005;112:80–83). Commercialization of new markers such as soluble vascular endothelial growth factors and placental growth factors for preeclampsia are also gathering some momentum.

More manufacturers have committed to and invested in resources for the development of age-related reference ranges. Even though informed consent requirements have become more daunting, as this goes to press there are several initiatives and studies underway to develop enhanced neonatal and pediatric ranges.

Finally, when this guideline was begun, tandem MS/MS was being performed in limited settings. With every year that has passed, more applications and capabilities for assaying not only novel, but routine, biomarkers have come into common practice.

SECTION I.

Maternal-Fetal Risk Assessment and Reference Values in Pregnancy

The ultimate objective of high quality maternal-fetal care is the uncomplicated birth of a healthy baby to a healthy mother at term. Both maternal mortality ratios and fetal mortality rates have plummeted in the industrialized countries. In the first decade of the 20th century, the maternal mortality ratio in the USA was 850 deaths per 100,000 live births, and fetal mortality was around 100 per 1,000 live births (*1*). By around 1995, maternal mortality in the USA was 7 per 1,000. In Canada, the total number of obstetric deaths/year from 1993 to 1997 was 4.4 per 100,000 live births. Infant mortality had dropped during this time to around 6–7 per 1,000 births in Canada and the USA. Similar improvements have occurred in most industrialized countries, but in many parts of the world, the maternal mortality ratio and perinatal mortality rate remain high.

As maternal and perinatal mortality has decreased in many countries, the focus of perinatal medicine has expanded to improving critical quality indicators for maternal and fetal health. Comprehensive national, state, or provincial perinatal surveillance systems have been introduced. These systems monitor a variety of indicators, including occurrence rates for specific select adverse occurrences, behavioral risk factors, and medical practices. These systems gather data that form a basis for sophisticated risk assessment and management programs for maternal-fetal health. An example of such a system is the Canadian Perinatal Surveillance System (*1*). Within these systems, the most important indicators of perinatal health are determined, a system of monitoring is instigated, and data are collected to provide a basis for assessing future intervention strategies.

Risk Assessment in Maternal-Fetal Health: Definitions and Principles

Risk management is a process whereby risk is defined and assessed in order that adverse outcomes may be prevented. Table I-1 defines terms used in risk management (*1*).

Table I-1. Definitions of Terms Used in Risk Management

Risk	An undesired event that has adverse consequences.
Risk impact	The loss associated with the risk (life, health, economic, social).
Risk probability	The likelihood that the event will occur (0 to 1).
Problem	When risk probability = 1, the risk is identified as a problem.
Risk exposure	Risk impact × risk probability (used to quantify risk).

Risk sources may be specific or generic (i.e., common to all pregnancies). For example, there is a generic *a priori* risk that a woman will have a multiple pregnancy. However, some families have a higher risk for multiple pregnancies. Following in-vitro fertilization, the risk of multiple pregnancies is also increased. There is thus a specific increased risk for multiple pregnancy over and above the generic or background risk. Risk exposure may be voluntary, such as the use of alcohol or illicit drugs, or involuntary, such as unanticipated exposure to an infectious agent.

Principles of Risk Management

1. Identify risk of adverse outcome.
2. Assess risk impact and probability.
3. Quantify the importance of risk.
4. Implement surveillance system for specific risks.
5. Identify cause or causes.
6. Identify modifiers or interventions.
7. Determine target risk reductions.
8. Implement interventions.
9. Utilize surveillance to evaluate efficacy of interventions.
10. Review and modify strategic approach as necessary to meet targets.

Evidence-Based Approach to Risk Reduction

Outcomes selected for risk reduction must be based on evidence that clearly demonstrates the efficacy of the reduction strategy. Levels of evidence-based assessment range from interventions with benefits clearly demonstrated by evidence from controlled trials, through those where evidence of benefit is strong though not established by randomized trials, to those in which there is insufficient evidence on which to base a recommendation. For other intervention strategies the balance between demonstrated benefits and risk of adverse effects must be carefully evaluated (*2*). Suggested interventions during pregnancy are discussed in later sections of this guideline.

GUIDELINE 1: Risk assessment intervention strategies.

Must be based on clearly demonstrated benefits through evidence from controlled trials or where evidence of benefit is strong though not established by randomized trials.

Maternal-Fetal Risk Assessment and the Laboratory

The laboratory role in risk management strategies varies with the strategy and the time of pregnancy in which it is important. Table I-2 indicates examples of such strategies.

Table I-2. Example of Laboratory-Based Gestation Specific Risk Reduction Strategies Decreasing Perinatal Risk in Diabetes

Time sensitivity	Intervention	Outcome objective
Pre-conception	Controlling blood glucose in known diabetics	Decrease risk of congenital abnormality
Second trimester 16 – 20 weeks	Maternal serum screen	Detect possible neural tube defect or other congenital anomalies
8 weeks	Glucose screen	Detect and manage abnormal glucose tolerance
Third trimester	Assess fetal lung maturity	Decrease neonatal respiratory distress syndrome
Post-natal	Monitor neonatal glucose, calcium	Prevent and manage neonatal metabolic problems

Maternal-Fetal Medicine and Reference Values

The laboratory is important as a generator of clinical pathology data on which risk assessment and management decisions are based. The marked physiological changes that occur as pregnancy progresses cause correspondingly marked changes in pregnancy reference ranges (*3–6*). Similarly great differences are seen in the fetus and the neonate, born at different stages of maturity. The range of different reference intervals that must be understood and accounted for in the pediatric and obstetric population is unlike that in a normal adult population. Maternal-Fetal and Pediatric Laboratory Medicine is therefore a laboratory service where utilization of reference values appropriate for gestation and developmental age assumes the greatest importance.

Although development and validation of reference intervals is an activity undervalued by funding agencies, method- and population-specific reference data should be a fundamental requirement for the interpretation of clinical data and for the institution of any risk management program. Published intervals can serve as a guideline, providing an indication of the magnitude and direction of change in reference intervals for a given analyte in a pediatric or obstetric population (*3–6*). However it is incumbent on laboratories testing specimens from these unique populations to validate their own reference data according to the specific methods used in their facilities.

GUIDELINE 2: Reference ranges.

Laboratories testing specimens from pregnant women or pediatric patients should develop or validate gestation- and age-specific reference intervals for every analyte offered.

SECTION II.

Vaccinations during Pregnancy

Vaccination of pregnant women poses theoretical risks to the fetus. Therefore, pregnant women should only receive a vaccine when the vaccine is unlikely to cause harm, the risk for disease exposure is high, and infection from the disease would pose a significant risk to the mother and/or fetus. When a vaccine is to be given during pregnancy, delay of administration until the second or third trimester, if possible, is a reasonable precaution to minimize concerns of possible teratogenicity. Potential risks to the mother include reactions to the vaccine that could compromise normal gestation and induce premature labor. Such events have not been observed in women immunized during the third trimester of pregnancy. When present, vaccine reactions have been limited to local injection site reactions (*7*).

GUIDELINE 3: Vaccination prior to pregnancy.

We recommend that women considering pregnancy have a healthcare professional review their immunization status and be given the option to be vaccinated prior to conception.

In the United States, women of childbearing age should already be immunized to measles, mumps, rubella, tetanus, diphtheria, and poliomyelitis as a result of childhood immunization. The only vaccines routinely recommended for administration to a pregnant woman in the United States are tetanus, diphtheria, and influenza (*7–11*). Pregnant women who have not received a diphtheria and tetanus toxoid (DT) booster during the previous 10 years should be given a booster dose. Pregnant women who are un-immunized or partially immunized should complete the primary series. Immunization of the pregnant woman with tetanus toxoid at least six weeks before delivery protects the newborn from tetanus neonatorum by stimulating the production of specific IgG antibodies that cross the placenta. Maternal immunization with tetanus toxoid worldwide has dramatically decreased the incidence of neonatal tetanus without evidence of adverse effects on the mother or fetus (*12*).

Women in the second and third trimesters of pregnancy and the early puerperium are at increased risk of complications and hospitalization from influenza (*13*). This risk is increased even in the absence of underlying risk factors. The Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention recommends trivalent inactivated influenza virus vaccine for all women who will be beyond 14 weeks of pregnancy during the influenza season, and for women with underlying high risk conditions regardless of their stage of pregnancy (*14*).

GUIDELINE 4: Vaccinations during pregnancy.

- Pregnant women who will be beyond 14 weeks of pregnancy during the influenza season should be vaccinated with the influenza vaccine.
- Pregnant women should be immunized with diphtheria and tetanus toxoid (dT) so they are protected prior to delivery.

Vaccines Indicated in Special Circumstances during Pregnancy

During epidemic or endemic situations, pregnant women can be immunized with vaccines against poliovirus (inactivated or live attenuated), hepatitis A, yellow fever, and meningococcus. Vaccines that can be administered during pregnancy to women at high risk include the hepatitis B and pneumococcal polysaccharide vaccine.

Poliovirus. Routine adult immunization with poliovirus vaccines is not recommended. However, pregnant women at high risk due to endemic or epidemic exposure can receive either oral polio vaccine or inactivated polio vaccine as recommended by the ACIP and the American Academy of Pediatrics (*15,16*).

Hepatitis. Hepatitis A and hepatitis B vaccines, if indicated, can be administered to a pregnant woman (*7–11*). Compared to adults, infants and young children who acquire hepatitis B infection are at increased risk for serious liver disease and even death due to hepatitis, chronic liver disease, and liver cancer. Vertical transmission of hepatitis B occurs in infants born to HBsAg-positive mothers with a 90% risk of developing a chronic infection without intervention. Pre-exposure immunization of susceptible individuals is the most effective means of preventing hepatitis B virus transmission. Risk factors that might indicate hepatitis B immunization of a pregnant woman include injection drug use, multiple sex partners, a job that exposes one to blood or body fluids, living with someone who is infected, or having sex with someone who is infected. The currently licensed recombinant DNA HBV vaccines containing HBsAg protein are safe and induce a long-lasting protective antibody response in greater than 90% of adults. Although safety data of these vaccines for the developing fetus are unavailable, no risk would be anticipated because the vaccines contain noninfectious surface antigen.

Vertical transmission of hepatitis A virus from mother to infant is rare. Post-exposure immunization with HAV vaccine is recommended in adults. Although safety data on pregnant women are limited, the risk to the fetus is considered to be low or nonexistent because the currently licensed vaccines in the United Sates contain inactivated, purified viral proteins obtained from HAV-infected human diploid fibroblast cell cultures.

Pregnancy is in general a contraindication to the administration of all live-virus vaccines. However, exceptions should be made when susceptibility and exposure are highly probable and the disease poses a greater risk to the mother and/or fetus than does immunization.

Yellow fever. Infection with yellow fever results in a mild to severe viral syndrome associated with high mortality. Immunization with live attenuated virus vaccine (17D strain) is recommended for all individuals nine months of age or older living or traveling to endemic areas or required by international regulations for travel to and from certain countries. In high-risk areas, women should have been immunized prior to pregnancy. Yellow fever vaccine may be administered to a pregnant woman who is at substantial risk of exposure to infection (such as might occur with international travel). Yet, it might be prudent to postpone travel until the infant is born, if possible, since one possible case of asymptomatic congenital infection was reported in an infant from Trinidad after maternal immunization during the first trimester (*17*).

Measles, mumps, rubella, etc. Measles, mumps, rubella, and varicella vaccines that are live-virus vaccines are contraindicated in pregnancy. However, because these diseases can cause significant illness in pregnant women and/or the fetus, every effort should be made to immunize susceptible women against these illnesses before they become pregnant *(7*). Women of childbearing age should wait at least three months after vaccination with these live-virus vaccines before becoming pregnant. Women who are pregnant but not vaccinated should get vaccinated following delivery. Evidence to date suggests that inadvertent administration of rubella vaccine to susceptible pregnant women rarely, if ever, causes congenital defects. The effect of varicella vaccine on the fetus is unknown.

Meningococcal. Pregnant women can be immunized with meningococcal vaccine when there is a substantial risk for infection, as during epidemics. The vaccine consists of purified bacterial capsular polysaccharides. Pregnant women immunized with a single dose of meningococcal vaccine had good antibody responses, transmitted the antibody through the placenta, and provided protection to the newborn infant during the first few months of life (*18*).

S. pneumoniae is the most common cause of invasive bacterial infection and otitis media in children less than five years of age. Maternal immunization against pneumococcus is an alternative strategy to protect young infants until they are able to produce an adequate response to active immunization, especially in high-risk groups. Pneumococcal polysaccharide vaccines administered to pregnant women during the third trimester of

pregnancy have been safe for pregnant women and their offspring and have transferred modest amounts of antibody to the infant (*19*).

During pregnancy, certain laboratory tests are performed routinely on all women to monitor the pregnancy. Some tests are done to diagnose problems while others are used as screening tests to determine risks of birth defects or of passing diseases onto the newborn. Tests may be obtained on samples from blood, urine, or the cervix. If problems are detected, then many may be treated during the pregnancy. In many states, some of these tests are required on pregnant women by law.

Syphilis. Syphilis is a sexually transmitted disease. All women should be screened serologically for syphilis early in pregnancy with a non-treponemal test (the Venereal Disease Research Laboratory Slide Test [VDRL] or Rapid Plasma Reagin [RPR]) and again at delivery (*20*). In areas of high prevalence and in patients at high risk for syphilis, an additional non-treponemal serum test should be performed at the beginning of the third trimester of pregnancy (week 28). During pregnancy, low-titer false positive non-treponemal antibody tests may occur. The non-treponemal antibody test should be confirmed as false positive with a treponemal antibody test (Fluorescent Treponemal Antibody Absorption Test [FTA-ABS]). When a pregnant woman has a reactive non-treponemal test result and a persistently negative treponemal test result, a false positive test is confirmed.

GUIDELINE 5: Screening for syphilis.

All pregnant women should be screened for syphilis early in pregnancy.

Rubella. Post-pubertal women without documentation of presumptive evidence of rubella immunity should be immunized, unless they are pregnant (*21*). Post-pubertal females should be advised not to become pregnant for three months following rubella vaccination. Routine prenatal screening for rubella immunity should be undertaken, and rubella vaccine administered to susceptible women during the immediate postpartum period before discharge.

GUIDELINE 6: Rubella immunization.

Routine prenatal screening for rubella immunity, and rubella vaccine administration to susceptible women, should occur three months prior to conception or immediately postpartum.

Hepatitis B virus. Serologic testing of all pregnant women for the hepatitis B surface antigen (HBsAg) is essential for identifying infants who require post-exposure immunoprophylaxis beginning at birth to prevent perinatal hepatitis B viral infection (*22*). In high-risk individuals, repeat testing may be indicated in the third trimester. Post-exposure immunoprophylaxis with hepatitis B immune globulin (HBIG) and the hepatitis B vaccine can substantially reduce the incidence of maternal-neonatal transmission of hepatitis B virus.

Hepatitis C virus. Seroprevalence among pregnant women in the United States is estimated at 1–2%. Maternal-neonatal transmission is estimated at 5%. Maternal co-infection with human immunodeficiency virus (HIV) has been associated with an increased risk of perinatal transmission of HCV (six-fold increase). Hepatitis C can lead to cirrhosis, hepatocellular carcinoma, hepatic failure, and death. Hepatitis C currently is the leading indication for liver transplantation in the United States. Both the American Academy of Pediatrics and the Centers for Disease Control and Prevention recommend that all children born to women who are infected with hepatitis C virus or have risk factors for infection be screened for hepatitis C (*23*). Most infected women are asymptomatic and unaware of their infection. The two major tests for the laboratory diagnosis of HCV infection are assays for HCV antibodies and assays to detect HCV nucleic acid (RNA). Diagnosis by antibody assays involves an initial

screening enzyme immunoassay (EIA). Repeated positive results are confirmed by a recombinant immunoblot assay (RIBA). Both assays detect IgG antibodies. Currently no IgM assays are available. PCR assays are used commonly in clinical practice in the early diagnosis of infection and to identify infection in infants when maternal serum antibody (IgG), which crosses the placenta, interferes with the ability to detect antibody produced by the infant. Universal testing of all pregnant women for hepatitis C may not be cost-effective currently. However, selective testing based on risk factors may be warranted.

GUIDELINE 7: Hepatitis B and C testing.

- Perform serologic testing of all pregnant women for hepatitis B by the hepatitis B surface antigen test.
- Perform serologic testing of pregnant women for hepatitis C by a screening enzyme immunoassay (EIA) if clinically indicated or requested.

Human immunodeficiency virus (HIV). HIV is the virus that causes acquired immunodeficiency syndrome (AIDS). More than 90% of infected children in the United Sates acquired their HIV infection from their mothers. A substantial decrease in recent years in perinatal AIDS is due to the successful intervention with zidovudine administered to HIV-infected pregnant women. It is recommended that all pregnant women be offered counseling and testing with consent for HIV (*24*). (Testing for HIV infection is unlike most routine blood testing because of risks for discrimination in jobs, school, and child care.) Adults develop serum antibody to HIV within 6–12 weeks after infection. Infants born to HIV-infected mothers have transplacentally acquired antibody and thus test seropositive from the time of birth. HIV nucleic acid detection by PCR of DNA extracted from peripheral blood mononuclear cells is the preferred test for diagnosis of HIV infection in infants. Infants born to HIV-infected mothers should be tested by HIV DNA PCR during the first 48 hours of life. Because of the possibility of maternal blood contamination, umbilical cord blood should not be used for testing. A second test should be performed at 1–2 months of age. A third test is recommended at 3–6 months of age. Any time a test is positive, a repeat test should be immediately obtained for confirmation. An infant is considered infected if two separate samples are positive. Infection can be excluded if two HIV DNA PCR samples are negative performed beyond 1 month of age and/or if one sample was obtained at 4 months of age or older (*24*).

GUIDELINE 8: HIV testing.

All pregnant women should have their HIV status evaluated by an appropriate antibody test after informed consent. Counseling must be provided regarding testing results.

SECTION III.

First Trimester Prenatal Screening and Diagnostic Evaluation

This section of the document will cover the utility of the 11- to 14-week scan, details of the nuchal translucency (NT) technique, the association between NT and chromosomal defects, combined screening methods for chromosomal defects, and the significance of an abnormal NT in the presence of a normal karyotype. Also covered are the integrated clinical, ultrasound, and laboratory management of ectopic pregnancy.

Utility of the 11- to 14-Week Scan

Confirmation of viability. It is self-evident that there is a role for a late first trimester ultrasound scan to confirm fetal viability. This has an important impact on maternal (and co-parental) well-being, especially when there has been a history of either recurrent pregnancy loss or subfertility treatment.

Accurate dating. Accurate dating of pregnancy can be invaluable in the presence of unsure dates or irregular menstrual cycles, particularly if difficult clinical decisions need to be made at the limits of fetal viability (23–24 weeks). The optimal timing of interventions such as prenatal diagnosis (biochemical, ultrasound, and invasive) or post-dates induction of labor requires knowledge of exact gestational age, which is most reliably determined by first trimester ultrasound. In the first trimester, transvaginal ultrasound is accurate within 4–7 days, as compared to more than 7–10 days at 18–20 weeks *(25).*

Diagnosis of multiples: amnionicity and chorionicity. First trimester ultrasound accurately diagnoses multiple gestations and reliably determines the number of chorions and amnions.

The determination of chorionicity is most accurate at 6–9 weeks' gestation, with a thick membrane or septum between gestational sacs present in multi-chorionic gestations. The lambda (λ) sign of a dichorionic pregnancy is best seen at 10–14 weeks. As gestational age increases, the dichorionic membrane becomes thinner, making the lambda sign less reliable after 16 weeks.

Monochorionic pregnancies are characterized by the absence of a septum, or the absence of a lambda sign. However, as the lambda sign in dichorionic pregnancies may disappear after 16 weeks, its absence is not diagnostic of a monochorionic pregnancy. Amnionicity in monochorionic pregnancies can be determined by the number of yolk sacs and visualization of the membrane (*26*).

Early diagnosis of major anomalies. Reports of diagnosis of anomalies by first trimester ultrasound include such defects as those of the central nervous system (CNS) (acrania/anencephaly); abdominal wall (omphalocoele); urinary tract (megacystitis); skeleton (caudal agenesis); and cardiovascular system (*26*).

Chromosomal Anomalies: Nuchal Translucency (NT)

Nuchal translucency (NT) is a sonolucent area in the nuchal region of the fetus observed in the first trimester, which normally resolves in the second trimester. Increased NT is associated with chromosomal aneuploidy, birth defects, and genetic syndromes (*25,27*).

The standard for NT. There is a set standard for NT for which certified training is required (26,27,28). The scan is performed at 11–13 weeks when crown-rump length (CRL) is 45–84 mm, by transvaginal (TV) or transabdominal (TA) ultrasound scan. The fetal position should be one of neutral flexion, and ≥ 75% of image is fetus in the mid-sagittal view, excluding amnion. Having located the point of maximum widening, the caliper is placed "on-to-on" (typical examples are shown in Figs. III-1 and III-2).

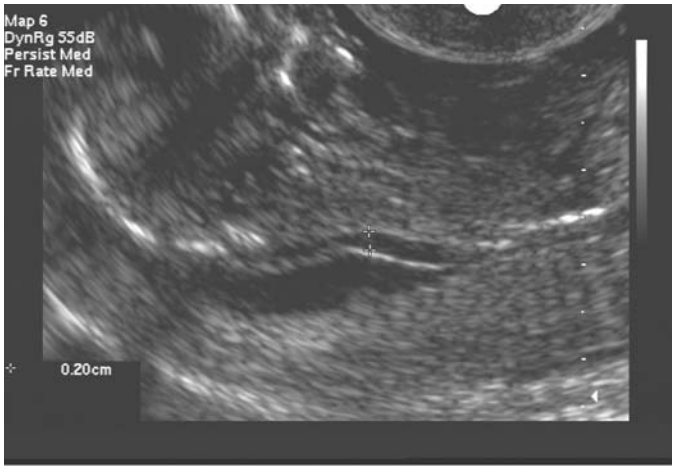


Fig. III-1. Normal fetus, NT 2.0 mm.

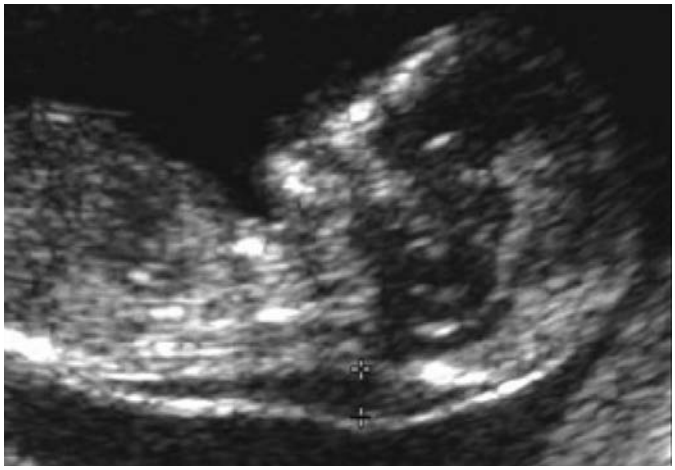


Fig. III-2. Trisomy 21 12 wk, NT 3.5 mm.

NT certification. This is undertaken through a three-stage process: first, a theoretical course (one-day course and qualification [MCQ] exam); then practical training with a log book of 50 images; finally, completion via an observed session (2-hour observed session or review video of 4 cases).

Ongoing quality assurance. After completion of certification, software is installed for risk assessment, and surveillance provided by a 6-month audit that includes a qualitative assessment of images and a quantitative assessment of the distribution of measurements within the site database.

Increased NT and aneuploidy. Increased NT is associated with trisomies 21, 18, and 13, triploidy, and Turner's syndrome (45 X0). The NT-adjusted risk combines a woman's background (age-related) risk and the NT measurement of the index pregnancy (Fig. III-3).

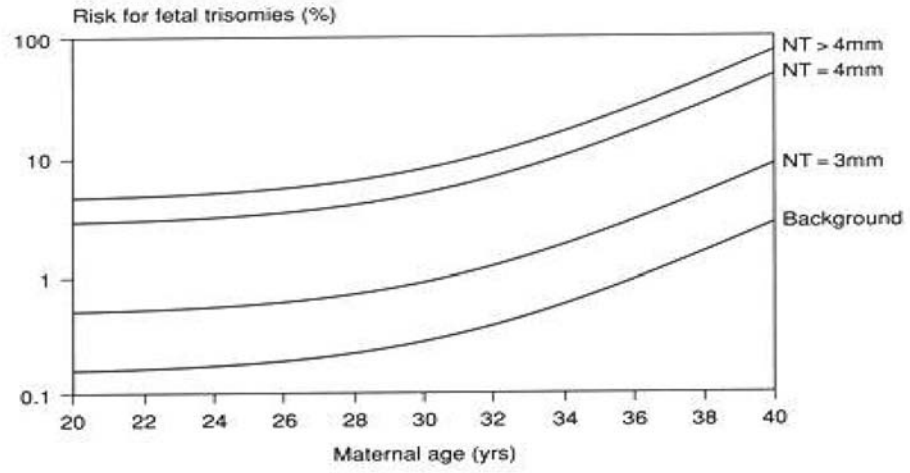


Fig. III-3. NT and risk for trisomy by maternal age (*27*).

Biochemical markers in the first trimester. The goal of including biochemical markers in the first-trimester screen is similar to that used in the triple (or quadruple) marker screen that has been widely accepted in the second trimester, namely to increase ascertainment of aneuploid fetuses without markedly increasing the false positive rate. The markers integrated in this approach are PAPP-A (pregnancy-associated plasma protein-A) and free β-hCG (*29,30*). This approach accounts for the impact of gestational age (PAPP-A increases and free β-hCG decreases with gestational age) (*29*).

Integration of NT and first- or second-trimester biochemistry. It is possible to combine the benefits of NT measurement with either first- or second-trimester biochemistry. Assuming a 5% false positive rate (accepted for age-based screening, which remains the gold standard across North America), Gilbert et al. (*25*) concluded that an integrated first and second trimester was the most cost-effective approach. However, there may be limited acceptability for women who wish to conclude their prenatal diagnosis as early as possible and for clinicians who may be asked to withhold an abnormal NT result pending biochemical results at 15 weeks (Table III-1).

NT, first trimester biochemistry, and other trisomies. In addition to screening for Trisomy 21 (Down syndrome), NT and integrated biochemistry are effective in screening for the other most common trisomies, trisomies 13 and 18 (*25,28,31*). Setting screen-positive limits at NT > 3.3 MOM (multiples of median), PAPP-A < 0.18 MOM, and free β-hCG < 0.28 MOM will detect 89% of Trisomy 18-affected pregnancies (*32*). For Trisomy 13, NT > 2.9 MOM, PAPP-A < 0.25 MOM, and free β-hCG < 0.51 MOM will lead to a detection rate of 90% (*30*).

What is the impact of first-trimester screening on the pregnancy loss rate following invasive prenatal diagnosis? Using advanced maternal age (AMA) ≥ 35y and the triple marker screen, it takes 60 amniocenteses to detect one Trisomy 21-affected fetus, at the cost of one normal fetus lost for every three Trisomy 21-affected fetuses detected. By employing an integrated first-trimester screen, 12 amniocenteses are required to detect one case of Trisomy 21, and one normal fetus is lost for every 15 cases of Trisomy 21 detected (*25,32*).

Table III-1.

The theoretical ("detection rate") and field ("reported rate") of screening for Trisomy 21, using a 5% false positive rate (*25*).

Procedure	Detection rate	Reported rate	Uptake	Process time (wk)
1st TM screening				
Maternal age	32%		80%	0
NT	74%	73%	80%	0
1st TM double test (PAPP-A, hCG)	63%	62%	80%	1
NT, PAPP-A, hCG	86%	80–85%	80%	1
2nd TM screening				
Maternal age	32%		80%	0
2nd TM double test (αFP, hCG)	60%	58–59%	80%	
Triple test (αFP, hCG, uE_3)	68%	67–69%	80%	1
Quadruple test (αFP, hCG, uE_3, inhibin A)	79%	76–79%	80%	1
Integrated test (1st TM: NT, PAPP-A; 2nd TM: quadruple test)	95%	94%	80%	1

What does an increased NT mean if the karyotype proves normal? In the presence of a normal karyotype, an increased NT is associated with an increased risk for an ever-increasing list of anatomical and genetic syndromes. Anatomical defects include cardiac defects, diaphragmatic hernia, omphalocoele, body stalk anomaly, fetal akinesia deformation sequence, and skeletal dysplasia (*28*). For example, among chromosomally normal fetuses, the risk of cardiac defects is 0.8 per 1000 pregnancies for fetuses with an NT < 5th centile for gestational age; the risk increases to 195 per 1000 for fetuses with an NT > 5.5 mm (*26*).

Genetic syndromes associated with an increased NT include Noonan syndrome, VACTERL, Zellweger syndrome, Joubert syndrome, Meckel-Gruber syndrome, and Nance-Sweeny syndrome (*28*).

Increased NT alone is not a fetal abnormality, but rates of miscarriage and perinatal death increase even when other structural or genetic anomalies are ruled out (*28*). Souka et al. (*28*) found that the rate of healthy live birth among fetuses with an NT 3.0–4.4 mm was 90%; with an NT 4.5–6.4 mm, it was 80%; and with an NT ≤ 6.5mm, it was 45%.

The fetal nasal bone—the next refinement of the 11- to 14-week scan? In an observational study, Cicero et al. (*31*) found that the absence of nasal bone was detected in fetuses with Trisomy 21 at 11–14 weeks of gestation. This observation requires confirmation in appropriately powered series in other centers.

The argument for making first-trimester screening the standard of care. The ultimate goal of prenatal interventions is to improve the risk assessment equation, by evaluating who would benefit the most and risk the least from invasive testing. There is evidence that NT integrated with either first- or second-trimester biochemistry improves the detection of aneuploidy and other fetuses at risk, while reducing the risks to normal fetuses from invasive testing, especially when compared with the current standard of care, age-based screening (*25–27,32*).

However, any screening program introducing this approach must meet accepted training and quality assurance standards. Quality assurance is crucial to maintain optimal detection rates while minimizing the false positive rate. The NT measurement should be performed in a technical setting that allows adequate time, and an NT measurement must never be approximated, as a bad image equals bad information. All screening programs need access to a computer program that integrates maternal age, ethnicity, and smoking status with gestational age, ultrasound, and biochemical findings to give a modified age-related risk.

This screening can be undertaken within the setting of an integrated first¯trimester clinic that coordinates the care of women presenting with threatened, inevitable, incomplete, and missed miscarriages (*33*); possible or confirmed ectopic pregnancy (*34*); and other first-trimester complications. Such integrated clinics utilizing clinical, ultrasound, and laboratory modalities are recommended by both the Confidential Enquiries into Maternal Deaths in the UK, the UK Royal College of Obstetricians and Gynecologists, and American experts (*34,35*). Early pregnancy assessment clinics have proven themselves to be cost-effective (*33*).

Ectopic Pregnancy

The management of ectopic pregnancy has improved incalculably since the simultaneous development of transvaginal ultrasound and rapid quantitative β-hCG measurement (*34,35*). The concentration of β-hCG should rise by more than 66% every two days in a viable intrauterine pregnancy. Once β-hCG is > 1,500 IU/L, an intrauterine fetal pole should be clearly visible transvaginally. Similarly, β-hCG > 15,000 IU/L should be associated with detectable fetal cardiac motion. The β-hCG must be interpreted in conjunction with ultrasound findings such as the presence or absence of an adnexal mass and/or free peritoneal fluid (blood). If no definite intrauterine pregnancy is observed, then pseudodecidualization must be considered. Heterotopic pregnancy (the presence of twin pregnancies, one intrauterine and the other extrauterine) must always be considered, particularly in the setting of assisted reproductive technology.

Medical treatment of ectopic pregnancy. This is achieved using methotrexate (a folate antagonist lethal to chorionic tissue), which is indicated in cases where the woman is hemodynamically stable, there is no intrauterine pregnancy detected by ultrasound, the ectopic pregnancy is < 4 cm diameter, and there is no evidence of rupture. Relative contraindications to methotrexate include visible fetal heart activity and a β-hCG > 10,000 mIU/ml.

Before administering methotrexate, the following investigations should be performed: CBC, renal, and liver

function tests. Methotrexate is administered at 50mg/m^2 body surface area intramuscularly as a single dose. Following methotrexate, the β-hCG may rise over the first three days, but by day seven there should be a minimum of > 15% fall in βhCG. If not, a repeat dose of methotrexate should be given following CBC, renal, and liver function tests. Once a response is noted, repeat β-hCG are performed weekly until negative. Using this approach, in subsequent pregnancies 87% are intrauterine, and only 13% are repeat ectopics.

Again, experts advise that this approach should be included within an integrated clinic, with established protocols (*34,35*). In this setting, cost savings ($US 3000 per treated patient), decreased morbidity, and improved patient satisfaction can be achieved.

GUIDELINE 9: First trimester testing.

- Hospitals providing obstetric and/or gynecological services should develop Early Pregnancy Assessment Clinics to streamline the care of women with diagnostic issues in the first trimester. These clinics should link obstetric, ultrasound, and laboratory services.
- Maternal age-based screening should no longer be accepted as the "gold standard" indication for invasive prenatal diagnosis, as it is associated with poor rates of detection of aneuploidy and with avoidable losses of diploid fetuses.
- Integrated, age-based, nuchal translucency and biochemical screening should be used to detect aneuploidy. Until the results of the randomized controlled trials are known, units should determine, based on the balance of evidence, whether to offer an integrated first-trimester screen or a two-step first (nuchal translucency, PAPP-A, and bhCG) and second (quadruple biochemistry) trimester screen.
- A fetus with an abnormal nuchal translucency, but found to be diploid, should be offered fetal echocardiography.
- A fetus with an abnormal nuchal translucency, but found to be diploid and with a normal detailed ultrasound anatomical screen, should be considered at increased risk for adverse outcomes, and be subjected to increased fetal surveillance for the remainder of the pregnancy.
- Nuchal translucency should be introduced only after appropriate training and certification, with access to the integrated computer programs, and continuing quality assurance.
- The safe and effective medical management of ectopic pregnancy is predicated on the coordinated efforts of obstetric, ultrasound, and laboratory services.

SECTION IV.

Second Trimester Prenatal Screening: Results from a Large Screening Program

More than two million women were screened for neural tube defects in a program established under the legislative authority of the California Code of Regulations. This section provides a history of that program and describes the results.

History and General Description of the California Expanded AFP Screening Program

The Genetic Disease Branch of the California Department of Health Services (GDB) began prenatal screening for neural tube defects through the measurement of maternal serum alpha-fetoprotein (AFP) in 1985 (*36*). More than 2.5 million women (2,621,849) were screened between 1985 and 1995. The analyte panel was expanded in 1995 to include two additional markers in maternal serum, chorionic gonadotropin (hCG) and unconjugated estriol (uE_3) in 1995 (*37*). With the addition of these markers, GDB also began a screening program for Down syndrome and Trisomy 18. From the beginning of the triple marker screening program to the end of 2001, more than 2.3 million women (2,329,429) were screened.

The screening program was established under the legislative authority of the California Code of Regulations, Title 17, Division 1, Chapter 4, Subchapter 9. As specified by the regulations, prenatal care providers must offer the screening program to women under their care between 15 and 20 weeks of gestation. Women then sign a document indicating that they consent to the screening, or they decline the screening. Women who consent to screening have a sample of blood drawn and sent for analysis. Individuals are charged a fee, which is paid by insurance or by MediCal.

The analysis is performed at one of eight regional laboratories[1] under contract with GDB. Upon arrival at the laboratory, the specimen is accessioned, including a determination whether or not the specimen is adequate for analysis.

Analyses are performed on multiple AutoDelphia Instruments (Perkin-Elmer Life Sciences, Boston, MA) using time-resolved fluorometry with reagents supplied by the instrument manufacturer.

The regional laboratories act under the direction of the central Genetic Disease Laboratory (GDL), which is responsible for quality assurance (QA). Daily results are monitored using prepared internal quality control materials at eight different concentrations, as well as by monitoring patient medians, analytical tray medians, and periodic external proficiency testing. Results that pass QA measures are released to the central computer for interpretation. Interpretation of the laboratory results depends not only on the analytical results from the laboratory but also on a number of demographic factors, the chief of which is gestational age. The typical values of all three analytes change during pregnancy (*38*). In order to create a common scale, values are converted into multiples of the median (MOM) by dividing by the population median for the given day of gestation. Thus, the overall median MOM should be 1.00. This MOM is further adjusted to take into account body weight (as a surrogate for blood volume) and ethnicity. For those women who are insulin-dependent diabetics, a further adjustment is required. Screening in twin pregnancies also requires a special adjustment.

[1]Northern Permanente Medical Group, Southern Permanente Medical Group, Western Clinical Laboratory, Allied Laboratories Inc., Fresno Community Medical Center, Orange Coast Regional Laboratory, Quest Diagnostics, Memorial Medical Center of Long Beach.

Screening for neural tube defects (and abdominal wall defects) is based on fixed cutoffs of the AFP MOM of 2.5 for singleton pregnancies and 4.5 for multiple gestations (*39*). This choice of cutoff results in a positive rate of approximately 1.5% and a detection rate in excess of 80% for the most common of these defects: anencephaly, (open) spina bifida, omphalocele, and gastroschisis.

Screening for Down syndrome is based on a risk estimate (*40*). The woman's age provides the *a priori* risk, which is adjusted based on the likelihood ratio of the analyte values in Down syndrome pregnancies compared to unaffected pregnancies. The resulting risk estimate is considered positive if the risk is greater than or equal to 1:190 at mid-trimester. This choice of risk cutoff gives an initial screen positive rate of 7–8%. Approximately one-third of the initial screen positives have overestimated gestational ages, giving falsely positive results. The detection rate of the screening program exceeds 60%. Since the population parameters in affected twin pregnancies are unknown, screening in twin pregnancies is performed by adjusting the analyte MOMs to the corresponding levels in a singleton pregnancy, and applying the risk algorithm.

Screening for Trisomy 18 is similarly based on risk. The *a priori* risk is again based on age; the risk of Trisomy 18 is approximately one-tenth the Down syndrome risk. Unconjugated estriol (uE_3) is a particularly important marker for Trisomy 18, so samples in which the uE_3 is considered invalid do not receive a risk estimate for Trisomy 18. Multiple gestations are also not screened for Trisomy 18. Further, affected fetuses are frequently subject to growth retardation, so no changes to the initial estimate of gestational age are permitted.

Positive results are called to the attention of the prenatal care provider by the staff of seven regional Expanded AFP Coordinator offices. The demographic data and other data upon which the positive result is based are confirmed by the Coordinator. Then the patient is offered a referral for diagnostic procedures to one of the State-Approved Prenatal Diagnosis Centers (PDC). There are currently 27 Comprehensive Prenatal Diagnostic Centers with 109 satellite offices throughout California.

At the PDC, the patient is offered genetic counseling, detailed ultrasound, and amniocentesis for diagnosis if indicated. The costs of these follow-up services are reimbursed by the Expanded AFP Screening Program to the Prenatal Diagnostic Centers from the fees collected for the screening.

This summary of the screening program provided the basis for the consideration of two significant points for evaluation and monitoring.

The Evaluation of Kit Lots (*41*)

The central laboratory (GDL) is also responsible for evaluation of new reagent lots. New kit lots are compared with the existing kit lot prior to use. This comparison is done using both quality control material and reference materials tested over a period of 4 days on 2 different instruments. Kit lots must match within 3% or the kit lot is referred back to the manufacturer for review and, if necessary, reformulation. Only kit lots that match within the 3% limits and exhibit acceptable precision of better than 5% are placed into use. The use of reference materials prevents the phenomenon of kit to kit drift since all lots are referenced against a known material. (See Table IV-1 for sample results of a kit lot evaluation.)

On occasion, an assay may have a new formulation. If preliminary quality assurance testing shows that the difference between the new assay and the current one will exceed 10%, then it is necessary to perform parallel testing with the two assays so that the new assay can be interpreted with appropriate medians, i.e., medians derived from that assay itself (Table IV-2).

It is also important to monitor the variation of the assay. Larger CVs lead to blurring the distinction between affected and unaffected pregnancies. The result is an increased false positive rate and a decreased detection rate.

Monitoring Medians and Positive Rates (Figs. IV-1 and IV-2)

At the population level, the fundamental outcome of the screening program is the screen positive rate, the percent of women who are identified for follow-up (*41*). Changes in this rate in either direction can be significant: too

high, and too many women are subject to invasive procedures, to say nothing of the increased cost of follow-up; too low, and too few affected fetuses are identified.

Further, monitoring the screen positive rate can point to the need for new adjustments. Early in the California Program's experience of triple marker testing, we observed a significant variation in the screen positive rates among the regional laboratories. Further analysis showed that the median estriol varied with time from blood collection. As a consequence, the Program instituted adjustments for small transit times and a policy of declaring estriol invalid when assayed more than eight days from blood collection.

Monitoring population medians is one tool for helping to identify why screen positive rates vary. One significant source of variation is ethnicity. We now adjust all three analytes for ethnicity—each in different ways—based on observed medians by ethnic group. It is important both to collect ethnicity information in the population and to monitor differences in medians in subgroups to provide the basis for an adjustment.

A second major adjustment is for maternal weight. Here, it is necessary to group the population in appropriately sized groups for the comparison. In many cases, deciles will suffice. An appropriate function (logarithmic or reciprocal) of the median analyte MOM in each group is regressed against the mean weight. The result gives a functional dependence that can be applied to the population generally.

Other factors from smaller segments of the population that lead to adjustments within the California program include diabetes and twins. In other programs there may be adjustments for smoking, previous history, number of pregnancies, and fertility assistance (*42*).

The median MOMs represent the center of the population distribution, but the screening cutoffs are far out in the tails of the distribution. Consequently, small changes in the median MOMs can be associated with large changes in the screen positive rates. Screening for Trisomy 18 is particularly sensitive to this phenomenon.

GUIDELINE 10: Screening assays.

Alpha-Fetoprotein [AFP] Assay

- The coefficient of variation should not exceed 5%. The accuracy should be within 3% from lot to lot.

Chorionic Gonadotropin [hCG] Assay

- The coefficient of variation should not exceed 5%. The accuracy should be within 3% from lot to lot.

Unconjugated Estriol [uE_3]

- The coefficient of variation should not exceed 7%. The accuracy should be within 5% from lot to lot.
- Since uE_3 is not stable on storage at room temperature, programs should monitor time from specimen collection to analysis and reject specimens that are old enough to exhibit deterioration.

The Screening Program

- Since all three analytes exhibit variation by race, data should be collected in order to adjust MOMs appropriately, if the required correction is greater than 10%.
- Repeat testing of initial positive results should not be performed because of the correlation of successive assays and the phenomenon of regression to the mean.
- Programs that do second-trimester screening should consider adding inhibin A and/or invasive trophoblast antigen [ITA], formerly known as hyperglycosylated hCG [HhCG].

Table IV-1. Validation of New Kit Lot

Interpretation Version	Start Date	Change of Medians	AFP Kit Lot	Kit Start Date	Number of Samples	Gestation		and Weight		and Ethnicity		Number of Samples	Gestation		and Weight		and Ethnicity	
						Median	log SD	Median	Log SD	Median	log SD		Median	log SD	Median	log SD	Median	log SD
6.00	7/29/95	X	111111	7/29/95	4399	1.03	0.1837	1.02	0.1745	1.01	0.1724	2419	1.02	0.1570	1.01	0.1495	1.00	0.1518
6.00			222222	8/8/95	51326	1.07	0.1830	1.05	0.1711	1.04	0.1722	28398	1.05	0.1601	1.05	0.1479	1.04	0.1472
6.01	11/6/95				25430	1.07	0.1793	1.05	0.1687	1.05	0.1674	15527	1.06	0.1578	1.06	0.1464	1.05	0.1479
6.01			222222-plate	12/21/95	15458	1.03	0.1798	1.02	0.1720	1.01	0.1713	9395	1.01	0.1575	1.01	0.1487	1.00	0.1472
6.10	1/18/96				75787	1.03	0.1817	1.02	0.1720	1.01	0.1706	47410	1.02	0.1575	1.02	0.1494	1.01	0.1472
6.10			222222-plate	5/22/96	12269	1.04	0.1797	1.02	0.1692	1.01	0.1685	7771	1.02	0.1664	1.02	0.1531	1.01	0.1524
6.10			333333-std	6/12/96	6442	1.04	0.1849	1.03	0.1791	1.03	0.1778	3976	1.03	0.1647	1.03	0.1537	1.02	0.1552
6.10			334333-tracer	6/22/96	9411	1.04	0.1817	1.03	0.1755	1.02	0.1773	5977	1.02	0.1671	1.01	0.1559	1.01	0.1552
6.11	7/10/96				33670	1.04	0.1868	1.03	0.1760	1.02	0.174	22052	1.03	0.1610	1.03	0.1486	1.02	0.1501
6.11			334333-plate	9/9/96	24498	1.05	0.1849	1.03	0.1742	1.03	0.1728	16685	1.04	0.1630	1.04	0.1501	1.03	0.1516
6.11			334343-tracer	10/23/96	57534	1.02	0.1823	1.01	0.1720	1.00	0.1731	40704	1.01	0.1599	1.01	0.1487	1.00	0.1502
7.00	2/7/97				30887	1.02	0.1810	1.01	0.1745	1.00	0.1731	22911	1.00	0.1601	1.00	0.1495	0.99	0.1503
7.00			334443-plate	4/3/97	7418	1.00	0.1777	0.99	0.1671	0.99	0.165	5539	0.99	0.1580	0.99	0.1480	0.98	0.1465
7.01	4/16/97				62491	1.01	0.1823	0.99	0.1724	0.99	0.171	47064	0.99	0.1601	0.99	0.1488	0.98	0.1473
7.10	8/12/97				26368	1.01	0.1843	1.00	0.1738	1.00	0.1724	21632	0.99	0.1608	0.99	0.1488	0.99	0.1480
7.10			334543-plate	10/2/97	68304	1.05	0.1843	1.05	0.1748	1.04	0.1735	56877	1.04	0.1624	1.04	0.1493	1.03	0.1501
7.10			444544-std	2/19/98	13393	1.06	0.1817	1.06	0.1700	1.05	0.168	11527	1.04	0.1587	1.05	0.1464	1.04	0.1457
7.10			444654-plate	3/16/98	31417	1.03	0.1791	1.03	0.1695	1.02	0.1681	26830	1.01	0.1561	1.02	0.1465	1.01	0.1458
7.10			445664-tracer	5/14/98	28091	1.05	0.1792	1.05	0.1680	1.05	0.1667	23985	1.04	0.1587	1.05	0.1464	1.04	0.1479
7.10			683741	7/9/98	3222	1.07	0.1799	1.08	0.1719	1.06	0.1719	2678	1.09	0.1602	1.10	0.1464	1.09	0.1450

Table IV-2. The Effect of Reformulation of the Unconjugated Estriol Assay

Week of Gestation	711530		717541		Percent Increase	
	LMP	U/S	LMP	U/S	LMP	U/S
15	0.84	0.85	0.89	0.91	5.95%	7.06%
16	1.04	1.06	1.13	1.15	8.65%	8.49%
17	1.32	1.33	1.45	1.46	9.85%	9.77%
18	1.61	1.61	1.78	1.78	10.56%	10.56%
19	1.9	1.89	2.14	2.17	12.63%	14.81%
20	2.19	2.13	2.49	2.44	13.70%	14.55%

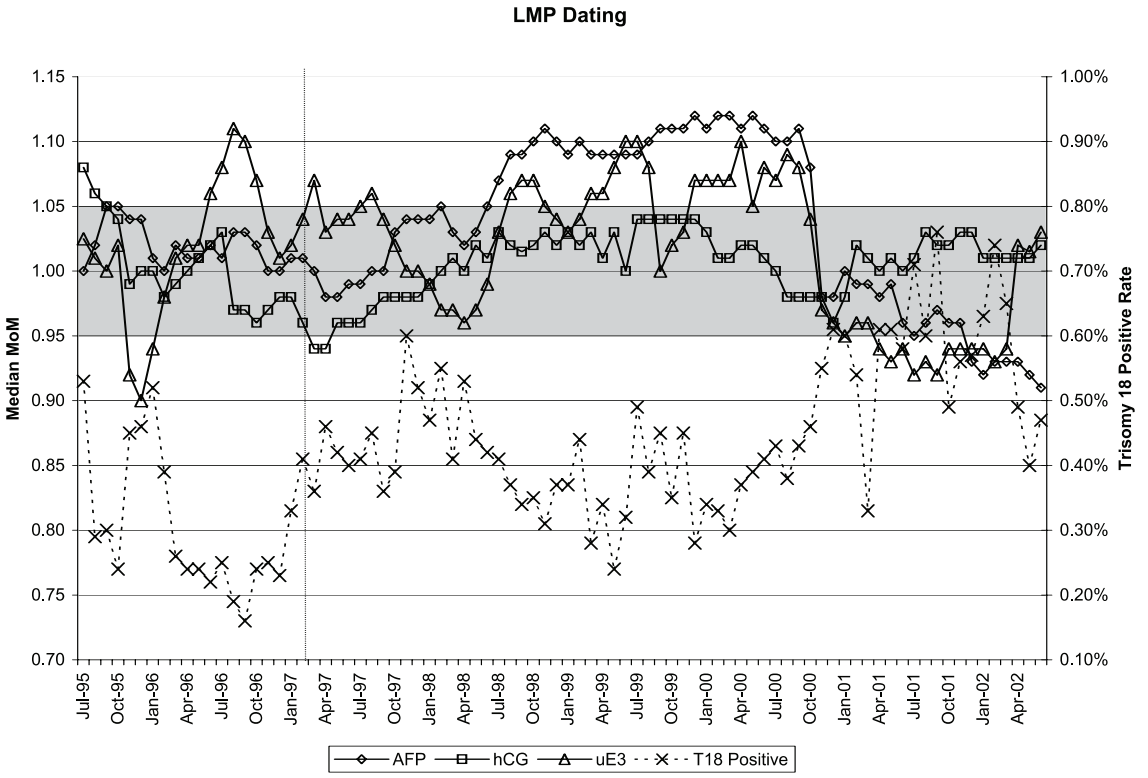


Fig. IV-1. Monitoring assay medians and Down syndrome screen positive rates. Data are shown for last menstrual period dating.

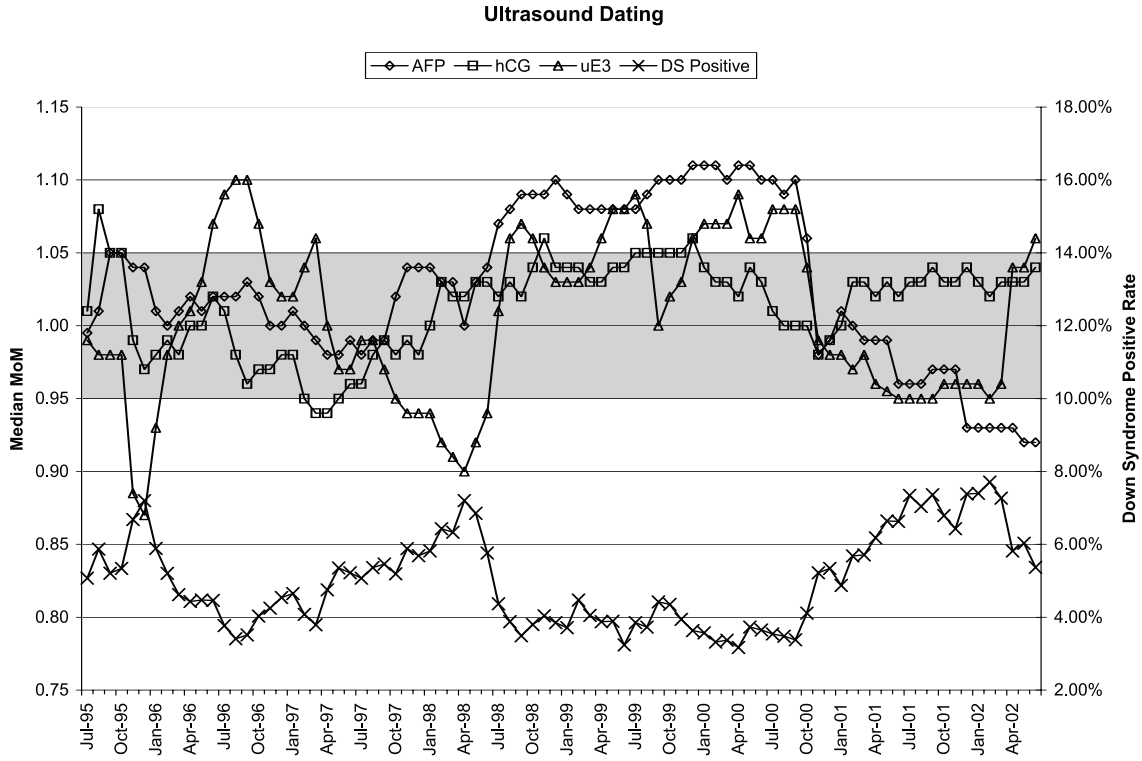


Fig. IV-2. Monitoring assay medians and Down syndrome screen positive rates. Data are shown for ultrasound dating.

SECTION V.

Follow-Up Diagnostic Assessment of the At-Risk Pregnancy

Pregnancies can be at risk for maternal reasons, fetal reasons, or both. These guidelines will focus on two maternal medical conditions: (1) the hypertensive disorders of pregnancy and (2) thromboembolic disease. This focus exists for the following reasons:

- These two medical conditions are among the most common medical disorders of pregnancy.
- They are the most serious of those disorders, as they are the most common causes of maternal death (*42*).
- The management of both conditions is focused on laboratory testing.
- The two conditions are increasingly interrelated in terms of pathogenesis and management.

The Hypertensive Disorders of Pregnancy

Hypertensive disorders complicate 5–10% of pregnancies worldwide, and remain a major cause of both maternal and perinatal mortality and morbidity in both developed and developing countries. The literature reveals a lack of consensus regarding how one should diagnose and classify the hypertensive disorders of pregnancy (HDP), and how one should manage them. This is due in large part to inconsistencies in terminology with respect to both the maternal HDP and the perinatal outcomes of interest. The lack of consensus is also due to the ill-defined relationship between the current classifications and the adverse maternal and perinatal outcomes that all clinicians and women wish to avoid.

Classification of HDP. Similar guidelines for the diagnosis and classification of preeclampsia have been produced by the Canadian Hypertension Society (CHS) (*43*), the U.S. National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (*44*), and the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP) (*45*), the latter two largely merged by the International Society for the Study of Hypertension in Pregnancy (ISSHP). All of these guidelines are based largely on expert opinion and all have their limitations.

First, most classifications are predicated on the occurrence of both hypertension and proteinuria. This fails to occur within the week prior to an eclamptic seizure in 40% of women (*46*). Therefore, in practice, the diagnosis of preeclampsia needs to be considered and excluded (by renal, hepatic, and hematological investigation) when either non-proteinuric gestational hypertension (present in 20% of women within a week of their first eclamptic seizure) or non-hypertensive gestational proteinuria (present in 10% of women) arises. Furthermore, in a secondary analysis of the National Institute for Child Health and Development (NICHD) aspirin trial for the prevention of preeclampsia, women who developed severe non-proteinuric gestational hypertension (vs. those who developed mild preeclampsia) had higher rates of both preterm delivery (< 37 weeks) and small-for-gestational-age infants (*47*). This reinforces the importance of considering the condition as other than one of pure hypertension and proteinuria.

Second, dichotomizing preeclampsia into mild or severe disease presumably differentiates women with lower risk from those with higher risk, but there are no shades of grey over a broad range of clinical situations.

Third, not accounting for gestational age in any of the current classification systems is a major problem. Gestational age is the most important predictor of both maternal and perinatal outcomes. Early-onset preeclampsia (< 32 weeks) is associated with a 20-fold higher risk of maternal mortality compared with preeclampsia that occurs at term (*48*), and is consistent with more perturbed neutrophil function and cytokine levels. Also, gesta-

tional age is the most important determinant of perinatal outcome among diploid fetuses (*49*). A greater than 50% chance of intact fetal survival in preeclampsia arises only when the delivery gestational age is ≥ 27^{+0} wk at a birth weight ≥ 600g (*50*).

Fourth, it is not known how aspects of the fetal syndrome of preeclampsia, which are identified by the CHS as adverse features, predict maternal risk.

Predicting preeclampsia. It is obvious that the current classifications of HDP are focused on diagnosing preeclampsia, because it is the most dangerous for both mother and baby. This is why so much of antenatal care is devoted to the detection of the disorder, and one of the primary reasons why women are seen every four weeks early in pregnancy, every two as pregnancy advances, and then every week for the last four to six weeks.

Preeclampsia is a multisystem disorder that has its roots in inadequate placentation (e.g., having an immunological basis) and/or excessive fetal demands (e.g., multiple gestation). When this mismatch is generated, an intravillous "soup" is released from the utero-placental circulation. This "soup" includes various inflammatory mediator (such as cytokines) and trophoblast fragments that produce maternal systemic inflammation and the well-documented endothelial cell dysfunction. The latter is thought to lead to the multiple organ system dysfunction of preeclampsia. This most commonly consists of hypertension and proteinuria, but may consist only of eclampsia, or liver enzyme abnormalities, for example. The Canadian guidelines are the only ones that attempt to account for the multiple organ dysfunction of preeclampsia, by including "adverse features" in the classification of the hypertensive disorders of pregnancy (HDP).

Proteinuria as an essential component of the classification of HDP. In the classification of HDP, proteinuria is key, but its diagnosis is problematic. Proteinuria is defined by the gold standard 24-hour urinary protein measurement of 0.3g/d or more.

In antenatal clinics, urinary dipstick testing (by visual inspection of dipsticks) is used because of its low cost and efficiency. This method is known to be neither sensitive nor specific, although sensitivity and specificity can be improved by using an automated device (*51*). In actual fact, the negative predictive value (NPV) of a negative or trace dipstick proteinuria in pregnancy is actually very good, exceeding 90% regardless of the method used.

The real problem is encountered for 1+ proteinuria, the positive predictive value (PPV) being less than 50% with use of automated testing. According to the existing classification systems, 1+ proteinuria should trigger the clinician to perform a 24-hour urine collection. Until the result is back, the management of that woman's pregnancy depends on how worried the clinician is. The situation is not much better when urinary dipstick testing reveals 2+ proteinuria, which has a PPV close to 50%. The clinician can be more certain about the presence of proteinuria with 3+ or 4+ proteinuria.

Given the uncertainties associated with interpretation of urinary dipstick testing, there has been enthusiasm for evaluation of urine protein:creatinine ratios, which compared with 24-hour urine collections are cheaper, easier for the patient to perform, and reportable to the clinician on the same day. With a cutoff of > 30 mg protein/mmol of creatinine, the PPV is at least 90% (*52*).

To complicate matters further, there is also evidence that the method of urinary protein analysis alters the quantification of urinary protein in a 24-hour urine collection, which is the "gold standard" (*53*). The benzoyl chloride assay, which is commonly used in hospital laboratories and is more sensitive to a complex protein mixture, has been found to be more sensitive than the Bradford assay, which is widely used in scientific laboratories and appears to be more specific. Protein assay specificity may be important, as albumin and transferrin are the principal components of proteins in less well-developed preeclampsia.

GUIDELINE 11: Recommended laboratory tests.

- The recommended laboratory tests for the diagnosis and evaluation of preeclampsia represent an amalgamation of those put forth by the Canadian, Australasian, and American groups, all of which differ in their specific recommendations. For example, the Canadian group recommends that urinary dipstick testing be abandoned, that tests of coagulation not be performed routinely (unless surgery and/or disseminated intravascular coagulation [DIC] is likely), and that serum albumin not be performed. However, the Australasians do recommend coagulation studies, as well as serum albumin testing given the inverse relationship between hypoalbuminemia and the risk of pulmonary edema.
- We surveyed Canadian practitioners and asked them whether or not they use the commonly recommended tests, and if so, how frequently they use them. Most reported using all of the blood tests and the urine tests at least once weekly. The exception (54) was daily use of urinary dipstick for proteinuria in women with suspected preeclampsia.
- What remains to be determined is exactly how the results of these tests, individually or in combination, relate to the risk of adverse maternal and perinatal outcomes. This awaits further study, and until such time, the recommended tests are based heavily on expert opinion.

Differential diagnosis of preeclampsia. The differential diagnosis of preeclampsia is that of underlying hypertension and/or other microangiopathies, such as thrombocytopenic purpura hemolytic-uremic syndrome, antiphospholipid antibody syndrome, sepsis/disseminated intravascular coagulation, vasculitis, or malignant hypertension. Also, preeclampsia must always be distinguished from the more ominous acute fatty liver of pregnancy, in which there is early liver dysfunction, characterized by an elevated INR and high bilirubin. Therefore, tests used to diagnose preeclampsia must include further testing if the history and the physical raise the suspicion of another disease process. Urinalysis may be particularly useful. In preeclampsia, the glomerular lesion of "endotheliosis" is not a proliferative one, and there should be no associated red blood cells (RBCs) or casts; RBCs should prompt consideration of associated placental abruption and/or another glomerular lesion.

Postnatal work-up of the woman who had preeclampsia. In this case, there are two issues to address: (1) ruling out underlying conditions that may have predisposed a woman to preeclampsia and may require or benefit from treatment (e.g., diabetes); and (2) identifying other cardiovascular risk factors, because having had an HDP increases long-term cardiovascular mortality and morbidity (55).

Risk factors for preeclampsia. Risk factors for preeclampsia include pre-existing hypertension and cardiovascular risk factors. Each of these is examined in turn, below.

Pre-existing hypertension. Follow-up beyond six weeks postpartum is necessary, recognizing that the hypertension of preeclampsia may take a few months to resolve. Persisting hypertension should be regarded as pre-existing, and as a condition that requires investigation of the patient's electrolytes, creatinine, urinalysis, TSH, calcium, and a plasma renin:aldosterone ratio. Fasting blood glucose will detect underlying diabetes, and a follow-up urinary protein to creatinine (or albumin to creatinine) ratio or 24-hour urinary protein (beyond three months postpartum) will detect persistent proteinuria suggestive of underlying renal disease. If preeclampsia was of early onset and severe, then thrombophilia testing is recommended, the details of which will be discussed below in the section, "Thromboembolism in Pregnancy."

Cardiovascular risk factors. After 6–12 weeks postpartum, when at least the majority of the physiological changes of pregnancy are resolved, it is appropriate to test for hyperlipidemia, hemoglobin A1C, and hyperhomocysteinemia. It is also appropriate to perform electrocardiography and echocardiography (to rule out left ventricular hypertrophy). For these women, as for women with gestational diabetes, pregnancy should be viewed as a "stress test" that they failed, and which has afforded them the opportunity to appreciate their increased risk of future cardiovascular health and to address it.

Thromboembolism in Pregnancy

Thromboembolism results from an interaction between environmental factors and patient factors (i.e., thrombophilia). It is well recognized that venous thromboembolic events (e.g., deep vein thrombosis [DVT]) are associated with thrombophilia, either genetic or acquired. However, it has been more recently appreciated that thrombophilia may be related to the following adverse placentally mediated events: early severe preeclampsia; severe intrauterine growth restriction (usually defined as birth weight < 5th or < 3rd centiles); stillbirth; or recurrent fetal loss (defined as three or more consecutive, unexplained miscarriages) (*56*). Although the association between thrombophilia and adverse placental events has not been entirely consistent, this may relate to different populations studied (e.g., Caucasians, 5% of whom carry the Factor V Leiden mutation), and variable definitions of outcomes. Therefore, thrombophilia testing may be required because of, for example, either a previous maternal DVT or a history of previous unexplained stillbirth.

Thrombophilia screening tests. Thrombophilia may be genetic or acquired. There has been a resurgence of enthusiasm for thrombophilia screening with the advent of newer tests that are able to reveal an abnormality in approximately 50% of individuals with thromboembolism or a family history of such.

Thrombophilia may result from a deficiency of anti-thrombotic factors (antithrombin, protein C, protein S); an increase in substrate (e.g., fibrinogen); abnormal coagulation proteins (e.g., Factor V Leiden mutation); or biochemical abnormalities (e.g., hyperhomocysteinemia). Although tissue factor pathway inhibitor is also relevant, problems with this in pregnancy haven't been described to date. Also, problems with the fibrinolytic system haven't been proven to be operative in patients with venous thromboembolic disease in or out of pregnancy.

When thrombophilia testing is performed in pregnancy, keep in mind that normal pregnancy is associated with an increase in procoagulant factors (e.g., Factor VIII) and a decrease in some anticoagulant factors (e.g., Protein S).

Other pregnancy-specific risk factors. There are other specific antenatal risk factors for thromboembolism in pregnancy:
- age of 35 years or more
- high gravidity
- obesity
- nephrotic syndrome
- diabetes
- gross varicose veins
- a current infection
- bed rest for more than four days prior to delivery
- preeclampsia (*57*).

The most common delivery risk factor, which is present in at least 20% of deliveries in North America, is Caesarian section; in the UK, thromboprophylaxis guidelines have been developed for women delivered by Caesarian section (*58*). Other "delivery" risk factors include pelvic trauma, immobility, and uterine sepsis.

Should all pregnant women be screened? Screening is not advocated for pregnancy alone, just as it is not advocated prior to taking the oral contraceptive pill, which is associated with a higher relative risk (RR) of thromboembolism than is pregnancy (*59*).

What should be done with the results of thrombophilia screening? If there is a history of a previous maternal event in pregnancy or on the pill, and the screen is negative, then the risk of recurrent DVT in pregnancy is approximately 2% and heparin thromboprophylaxis may be safely withheld (*60*). If however, there is a family history of clot and/or there are one or more abnormalities on screening, then opinion favors thromboprophylaxis with heparin. Whether or not the latter is effective in preventing recurrent DVT in pregnancy, which may occur in up to 16% of women, is based on extrapolation of effectiveness from the surgical thromboprophylaxis literature.

If there is a history of a previous "placental event," then the presence of an antiphospholipid antibody and a history of recurrent miscarriage warrant treatment with low-dose aspirin (81mg/d) and low-dose heparin (unfractionated or low molecular weight) (61). Although observational literature suggests that heparin prophylaxis may be effective for other thrombophilias and a history of other "placental events," this remains to be proven by randomized controlled trials.

In summary, the thromboembolic disorders are of importance to both mother and fetus. How to manage women at increased maternal and/or fetal risk is very unclear. Experts agree on providing prophylaxis for symptomatic thrombophilia (especially multiple thrombophilias); "symptomatic" is defined as a maternal thromboembolic event. With respect to pregnancy (or placental) events, women with the specific thrombophilia of antiphospholipid antibody syndrome and the placental event of recurrent fetal loss are the only ones for whom there is good (but not sufficient) evidence to recommend thromboprophylaxis in future pregnancy.

SECTION VI.

Laboratory Medicine Practice Guidelines for the Evaluation of the High-Risk Pregnancy

Clinicians commonly classify pregnancies as low- and high-risk. Many causes contribute to the high-risk classification (Table VI-1).

Table VI-1. High-Risk Pregnancies

- Preterm birth
- Premature rupture of membranes
- Twins and higher multiples
- Isoimmunization disease
- Liver disease
- Preeclampsia (including HELLP)
- Fetal anomalies
- Infections (e.g., Group B streptococcus, HIV)
- Maternal conditions (e.g., Graves)

Although a number of medical conditions can affect the patient who has a high-risk pregnancy, these laboratory medicine guidelines review two high-risk pregnancy topics: (1) preterm birth and (2) fetal lung maturity.

Preterm Birth

Normal human gestations endure approximately 40 weeks. A "preterm birth" is defined as a delivery of the infant prior to 37 weeks' gestation. Births before 32 weeks' gestation are classified as "very preterm birth." In addition to being classified by their "gestational" age, newborns can also be classified by birth weight. Any infant < 2500 g is classified as "low birth weight" (LBW), and those < 1500 g are classified as "very low birth weight" (VLBW). Preterm birth and LBW are the most common of the high-risk pregnancies. Although the incidence of very preterm birth in the U.S. from 1981 to 2000, has been constant at 1.9%, the incidence of preterm birth has increased from 9.4% to 11.6% (62).

A variety of obstetrical and maternal conditions precede preterm birth (Table VI-2). Some of these conditions are causative and others are merely associations.

Table VI-2. Conditions Associated with Preterm Birth

Obstetrical	Maternal
Preterm labor	Previous preterm birth
Preterm ruptured membranes	Diabetes
Preeclampsia	Asthma
Abrupta placenta	Drug abuse
Multiple gestation	Pyelonephritis
Placenta previa	Maternal race (higher in African Americans)
Fetal growth retardation	Poor nutrition
Excessive or inadequate amniotic fluid volume	Low pre-pregnancy weight
Fetal anomalies	Inadequate prenatal care
Amnionitis	Strenuous work
Incompetent cervix	High personal stress
	Anemia
	Tobacco use
	Infections

Preterm birth is categorized as "spontaneous" or "indicated." Spontaneous preterm birth is more frequent, accounting for about three-quarters of the cases, and occurs unplanned. The causes of spontaneous preterm birth include preterm labor, preterm premature rupture of membranes, amnionitis, and incompetent cervix. In one-quarter of preterm birth cases, the mother or fetus has a disorder, such as preeclampsia or fetal distress, that will improve following delivery. The clinician may therefore elect an early delivery in these cases to improve both maternal and fetal outcomes. These preterm births are therefore "indicated."

Even though the preterm birth rate has worsened, the infant morbidity and mortality rate for the preterm birth has improved. In 1980 the infant mortality rate in the United States was 12.6 per 1,000 live births. This declined to 6.9 per 1,000 live births in 2000 (63). Morbidity following preterm birth includes respiratory distress syndrome (RDS), bronchopulmonary dysplasia, intraventricular hemorrhage, patent ductus arteriosus, necrotizing enterocolitis, and sepsis. Most preterm infants have extended hospital stays.

Tests for predicting preterm birth. Several tests have been advocated to predict preterm birth, including fetal fibronectin (fFN), cervical length by ultrasound, salivary estriol (Sal-Est), alkaline phosphatase, maternal serum alpha-fetoprotein, and granulocyte colony-stimulating formation (Table VI-3). Fetal fibronectin and salivary estriol are addressed specifically in this section.

Fetal fibronectin (fFN). Fibronectin is adhesive glycoprotein that cross-links collagen to bind cells together. The fetal form has a unique epitope. Labor increases fFN in cervical and vaginal secretions. The specimen is obtained by collecting vaginal secretions in a specially designed Dacron swab. When fully saturated, the swab contains approximately 150 µL of secretions. Immunoassay is used to measure fFN; vaginal secretion fFN concentrations < 50 ng/mL indicate that delivery is not imminent. (Amniotic fluid is rich in fFN, therefore the test cannot be used in women with ruptured membranes.) The FDA has approved fFN for the diagnosis of impending premature delivery in symptomatic women who are at 24.0 to 34.9 weeks' gestation. For asymptomatic women who are at 22 to 30.9 weeks' gestation, fFN is FDA approved for predicting the risk of preterm delivery. In this group, the positive predictive value is a low 13%, but the negative predictive value is high at 99.5% (*64*).

Half of mothers thought to be in preterm labor deliver at term without treatment. Without fFN testing, 20% of those sent home deliver preterm. For predicting delivery within 7 days, in symptomatic women, studies (*65–68*) have determined the fFN test's sensitivity to be 57–93% and specificity to be 73–92% in women at 24 to 34.9 weeks' gestation. The positive predictive value has varied from 9% to 29%, whereas the negative predictive value is much higher at 97–99.6%.

Using fFN as a test to overrule the medical decision to admit a suspected preterm labor patient to the hospital appears to be cost-effective (*69*). The baseline costs were estimated in women without the use of fFN. If fFN is used in women with mild preterm labor symptoms, more than twice as many would be admitted to the hospital, nearly doubling the cost. When used to exclude admissions in women with more significant preterm labor symptoms, fFN would decrease costs by about 18%. Thus, proper use of fFN is necessary to prevent unnecessary admissions and increasing healthcare costs.

GUIDELINE 12: Use of fetal fibronectin (fFN).

- Use of fFN to veto the decision to admit a symptomatic patient to the hospital who is thought to be at 24.0 to 34.9 weeks' gestation is cost effective.
- Although some studies report that fFN might be useful in predicting which women could have labor induced therapeutically, more outcome studies are needed to determine the cost benefit of this use.

Use of fFN has been proposed at term (38 to 42 weeks' gestation) to predict probability that delivery can be induced (*70*). Most clinicians rely on the Bishop score (*71*) or cervical dilatation (*72*) for this assessment.

Salivary Estriol (Sal-Est). Estriol is a steroid hormone made by placenta from 16α-hydroxyl dehydroepiandosterone sulfate (16α-OH DHEA-S). This intermediate requires functioning fetal liver and adrenal glands. Estriol is excreted in milligrams per day and rises throughout pregnancy. Salivary estriol reflects unbound, unconjugated serum estriol and is approximately 1.00 ng/mL at 30 weeks and 3.00 ng/mL at term. Salivary estriol surges about five weeks prior to delivery (*73*). Salivary estriol is "still under assessment and should not be used outside of research protocols" (*74*).

GUIDELINE 13: Salivary estriol.

There is insufficient evidence to recommend the routine use of salivary estriol during pregnancy.

Predicting spontaneous preterm birth. A large multicenter trial (*75*) termed the Preterm Prediction Study was conducted to identify a population at risk for preterm birth. Twenty-eight biologic markers were included. The study subjects were asymptomatic at 23–24 weeks' gestation. The outcomes were preterm delivery at < 32 and < 35 weeks' gestation.

Table VI-3. Predictors of Preterm Birth < 35 wks*

Predictor	Odds Ratio
Fetal fibronectin (> 50 ng/mL)	6.6
Alkaline phosphatase (> 90th percentile)	4.0
History of preterm birth	4.0
Cervical length (≤ 25 mm)	3.9
Maternal serum alpha-fetoprotein (> 90th percentile)	3.9
Granulocyte CSF (> 75th percentile)	3.1

*From reference 75.

GUIDELINE 14: Preterm birth interventions in asymptomatic women.

Preterm birth interventions are not effective in asymptomatic women. Therefore tests such as fFN and salivary estriol that predict preterm birth in this group are not useful outside the research setting.

Fetal Lung Maturity (FLM)

Neonatal Respiratory Distress Syndrome (RDS) is a common disorder of preterm infants and infants with delayed maturation, such as those born to poorly controlled diabetic mothers. This disorder is caused by a deficiency of surfactant. Treatment has improved dramatically and requires increased oxygen and mechanical ventilation. Treating the newborn with exogenous surfactant at birth can often ameliorate the symptoms.

The lungs make surfactant in the form of lamellar bodies (LB) inside Type II pneumocytes. These hydrophobic structures are 1–5 microns in diameter (*76*) and contain surface-tension-reducing phospholipids and three specific proteins, SP-A, B, C, and D (*77,78*). The LB are excreted by exocytosis, and in the aerated lung, unravel to coat the air surface interface. Production starts as early as 28 weeks' gestation, but there is a surge in production at about 36 weeks for most fetuses. The newborn lung contains about 100 times more surfactant per lung volume than the adult lung.

The phospholipid content of LB is mostly lecithin (phosphatidylcholine [PC], phosphatidylinositol [PI], phosphatidylglycerol [PG], and phosphatidylethanolamine [PE]), but little, if any, sphingomyelin (S). Low PC concentrations are present in amniotic fluid up to 36 weeks' gestation, when production surges. PG production starts at this time in most normal pregnancies (*79*).

Testing for FLM has declined over the past 10 years. Surfactant therapy and clinical adherence to obstetrical guidelines have lessened demand. The clinician uses FLM results to assess whether best infant survival will be achieved in utero or following an early delivery. Knowing that fetal lung is producing adequate surfactant sways the decision toward delivery. The clinician can delay delivery by using tocolytic drugs such as ritodrine, can accelerate fetal surfactant production by administering steroids to the mother and delivering after three days, and can enhance labor and early delivery with the use of pitocin.

FLM testing is not indicated in normal pregnancies if the gestational age is accurately known to be at least 36 weeks (80). The best evidence for determining fetal maturation is an early positive pregnancy test at least 36 weeks in the past. Fetal heart tones for 20 weeks, or ultrasonographic evidence of a fetal heartbeat for 30 weeks, are also good indicators of a fetus that is old enough to have achieved pulmonary maturity. Also useful is ultrasound dating at 6 to 11 weeks' gestation that supports a pregnancy of at least 39 weeks, or measurements at 12–20 weeks' gestation that confirm a pregnancy of at least 39 weeks.

An ideal FLM test would be an imaging test available at the bedside. Such a test does not exist currently. All valid tests require analysis of amniotic fluid. This is best collected by amniocentesis even in the presence of ruptured membranes. The method should be available in most laboratories, not affected by blood or meconium, with results available rapidly at any time. False mature results have more dire medical consequences than do false immature results, because the former may tip the scales toward an unwarranted early delivery decision.

Tests for predicting fetal lung maturity. Available tests for predicting fetal lung maturity are listed in Table VI-4.

Table VI-4. Use of Fetal Lung Maturity Testing Methods (2002)*

Method	Source	Number of Laboratories [a]
Surfactant/albumin ratio (TDx FLM II)	Abbott Laboratories	462
Phosphatidylglycerol (AmnioStat-FLM)	Irving Scientific	447
Lecithin/sphingomyelin ratio	Helena Laboratories, and "laboratory developed test"	138
Phosphatidylglycerol (1-dimensional TLC)	"laboratory developed test"	92
Phosphatidylglycerol (2-dimensional TLC)	"laboratory developed test"	18
Lamellar body counts (LBC)	"laboratory developed test"	59[b]
Foam stability	"laboratory developed test"	< 50[c]
Fluorescence polarization (NBD-PC), Fpol	"laboratory developed test"	9

*Modified from Gronowski AM, ed. *Handbook of Clinical Laboratory Testing during Pregnancy*. New York: Humana Press, 2004:58.

[a]Data from reference 81, unless stated otherwise.

[b]Data from reference 82.

[c]Author's estimate.

All FLM tests have high (~95%) sensitivity, having immature results in RDS cases. All also suffer from mediocre (~65%) specificity, yielding mature results in most, but not all, cases with adequate fetal pulmonary development. The clinical outcome studies of L/S ratio (83–87); Fpol (83,88–90); TDx FLM II (91,92); and LBC (85,93–103) have very similar results in clinical outcome studies. The quantitative results of these tests reveal the degree of pulmonary maturation and are therefore more prognostically useful to the clinician than the qualitative tests. The foam stability test (104–109) is no longer available commercially and is used as a "laboratory developed test" by few laboratories. The turn-around time for L/S ratio is much greater than the other tests. Many laboratories have switched from L/S ratio testing to one of the rapid tests. The AmnioStat-FLM (110–114) is a qualitative test offered by more than 400 laboratories, but in late 2003, the manufacturer was unable to supply reagents, making this test unavailable for at least four months.

Although there are no controlled trials evaluating the cost-effectiveness of using an FLM test, there are many studies, cited above, on their effectiveness with respect to predicting fetal pulmonary status. Even though this use is decreasing, many FLM test requests remain.

GUIDELINE 15: Fetal lung maturity (FLM) testing.

- Fetal lung maturity (FLM) testing should be available in hospitals that deliver infants. The FLM test should be available routinely once per day and in urgent settings within an hour of specimen submission.
- The choice of rapid test depends on patient population:
  - Low risk population: qualitative PG (AmnioStat-FLM); TDx FLM II; LBC; or Foam Stability
  - High risk population: TDx FLM II; F Pol
- If a rapid FLM test is available, referral of L/S ratio requests to another laboratory is acceptable practice.

Predictive value. Because of the high sensitivity and low prevalence of RDS, the predictive value of a mature result is very good, about 98%. Conversely, the poor specificity and low prevalence produces a poor predictive value of an immature result. For example, analysis of 488 cases at the University of Utah from 1988 to 1993 showed that 43 of 135 infants with an immature L/S developed RDS. Thus, the predictive value of an immature L/S in this setting is about 32% (note that while sensitivity and specificity can be applied to different settings, predictive value cannot—it depends on the prevalence of RDS). During this same time period, 7 of 353 infants with a mature L/S developed RDS. Thus the predictive value of a mature L/S is 98% in this setting.

The manufacturer of TDx FLM II (Abbott Laboratories) recommends three interpretation categories: immature (≤ 39 mg/g); intermediate (40–54 mg/g); and mature (≥ 55 mg/g). Clinical outcome studies indicate that the upper mature limit could be safely lowered (91,92). Therefore, contrary to the manufacturer's recommendation, an upper decision limit of ≥ 45 mg/g should improve specificity to about 85% while maintaining sensitivity at > 95%.

GUIDELINE 16: TDx FLM II

Use of 45 mg/g as the maturity decision threshold for TDx FLM II reduces the number of false immature results without adversely increasing the number of false mature results.

LBC tests are rapid, but the results differ by instrument (93). LBC maturity thresholds vary dramatically (19,000–50,000/μL) and are caused by using different centrifugation protocols (115) and different analyzers (116).

GUIDELINE 17: Laboratories using LBC for FLM should determine reference values by doing the following:

(A) performing a clinical outcome study
(B) comparing their method to a method used in an outcome study using paired amniotic fluid specimens and then adjusting the threshold or transforming the results to agree with the primary method.

- Laboratories using LBC for FLM should not use centrifugation in order to improve precision.

Blood contamination. Compared to amniotic fluid, blood contains a high concentration of phospholipids. Therefore, the FLM results of bloody amniotic fluid specimens are altered. The exception is PG (117,118). Even though blood contamination alters the FLM results, it does not produce false mature results (119).

GUIDELINE 18: Blood contamination.

Bloody amniotic fluid specimens should be tested for FLM. Mature and very immature results are trustworthy, whereas borderline immature results could be falsely low or high.

Meconium contamination. The presence of heavy meconium contamination (> 15 g/dL) interferes with most FLM results (120). Moderate meconium contamination (about 5 g/dL) can produce erroneous L/S ratio values from immature to mature (121,122) and interferes with fluorescence polarization methods (TDx FLM II and FPol) (123), but does not cause false mature AmnioStat-FLM PG results (117,118). LBC results increase by less than 5000 particle/µL with light contamination (94).

GUIDELINE 19: Meconium contamination.

Amniotic fluid specimens contaminated with moderate meconium (greater than 5 g/dL) should not be tested for FLM except by using PG.

Diabetes. Tight glycemic control and new treatment algorithms have significantly reduced the incidence of RDS in the diabetic pregnancy (124,125). While some reports indicate more RDS cases despite mature L/S ratio results (126), others indicate no additional risk (127,128). For poorly controlled diabetes, the gestation at which the L/S ratio begins to rise has been reported to be both delayed (129) and not delayed (79) as compared to non-diabetic pregnancies. In recent studies of well controlled diabetics, agreement exists that the time of the L/S ratio surge is not affected by diabetes (79,130). Several TDx FLM II studies have shown that these results are reliable when used in diabetic pregnancies (131–133).

Regardless of degree of diabetic control, the detection of PG is delayed about 1.5 weeks (*79,134,135*). Even though many clinicians rely exclusively on PG for management of diabetic patients, there are no modern studies to support this practice.

There are insufficient outcome studies to evaluate the effect of diabetes on LBC and foam stability.

GUIDELINE 20: Effect of diabetes on reference values.

- In diabetic patients, separate reference values are not required for TDx FLM II, FPol, L/S ratio, and PG.
- There are insufficient studies to evaluate the effect of diabetes on LBC and foam stability test reliability.

Twin pregnancy. RDS is a frequent complication in twin pregnancy and can be discordant, especially prior to 31 weeks (*136*).

GUIDELINE 21: Twin pregnancy.

If testing for FLM prior to 32 weeks' gestation, both sacs should be sampled.

SECTION VII.

Current Practices and Guidelines for Evaluation of the Newborn Infant

With advances in modern medicine, the survival rate of newborns weighing < 1000 g has improved from about 0.5% in the 1940s, to over 60% by the 1990s. However, for the tiniest of infants, those weighing < 500 g, the survival rates remain about 6% (137).

The causes most frequently associated with neonatal mortality include infection, pulmonary complications, CNS damage, renal damage, and water/electrolyte imbalance.

The predominant reasons for admission in the Neonatal Intensive Care Unit (NICU) are preterm deliveries and birth weight < 1000 g. Such infants often require respiratory and circulatory support, and many have also undergone major emergency surgery.

As the newborn makes the transition from total maternal and placental dependency to independent metabolism, many biochemical markers adjust from values similar to the mother's circulation to values more reflective of the newborn's own metabolism (138). In the newborn, both fat content and water content differ from values seen in older infants. Water content in a full-term infant may be 20% higher; fat content is a function of gestational age, ranging from about 3.5% in a baby born at 28 weeks to approx 15% in a full-term baby. In premature infants immature liver function can cause a slower rate of metabolism and drug excretion, thus making such infants more susceptible to drug toxicity.

Age-Specific Reference Ranges

The issue of reference ranges is a challenge for pediatricians in general and for neonatologists in particular. Age-specific reference intervals are critical for appropriate interpretation of test results. Due to the growing number of preterm babies, the need becomes even greater for age-related gestational and postnatal reference ranges. Since "normal" ranges cannot be applied to preterm, and since obtaining informed consent for specimens is increasingly difficult, laboratories are dependent upon published reference ranges and validating these ranges as best as possible. Many of the published reference intervals are defined for specific methods and specific instruments. Results should be interpreted carefully based on the method and the instrument used. For proper interpretation of results, clinicians must be aware of circumstances where reference intervals for gestational age are not available or where adult reference intervals are used (Table VII-1).

As indicated in Section 1, age-related gestational and postnatal reference ranges should be used as available.

Table VII-1. Typical Comparison of Common Markers in the Adult with Those of a Full-Term Infant (*139*)

Note: reference intervals will vary, depending on instrument and method used.

Analyte	Adult Range	Full-Term Newborn
Albumin, g/L	37 – 56	26 – 36
Total protein, g/L	63 – 85	34 – 70
Alkaline phosphatase, mK/L	0.8 – 2.9	0.8 – 6.7
Ionized calcium, mmol/L	1.2 – 1.33	1.2 – 1.5
Uric acid, mmol/L	0.11 – 0.30	0.18 – 0.51
Thyroxine, nmol/L	63 – 129	75.9 – 277
Total bilirubin, µmol/L	< 17.1	< 205
CKMB, %	< 2.0 %	1.5 – 8.0%
Phosphorus, mmol/L	0.90 – 1.45	1.45 – 2.58
Ammonia, µmol/L	< 35	< 50

Alkaline phosphatase in infants is higher due to rapidly forming bone structure. Thyroxine has an upper reference range of 277 mmol/L, which declines rapidly during the first couple of hours and drops to adult levels within the first few days. Total bilirubin is typically higher due to immature liver function.

Because of these differences, it is crucial for the laboratory to provide the appropriate reference ranges, as some values change hourly during the first three days into the first month.

GUIDELINE 22: Neonatal reference ranges.

The laboratory should provide expected ranges relative to adult levels for neonates.

Preanalytical Issues

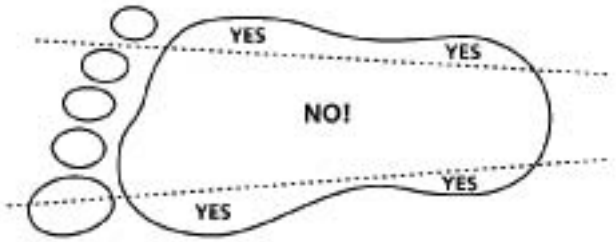
Collection of specimens. While specimen collection on tiny and ill infants can be labor intensive, it is critical to the management of NICU laboratories to have dedicated individuals who are well trained in collecting these specimens.

Metabolic diseases. Pediatric laboratories should have ready access to reference laboratories that have the equipment needed for analysis and monitoring of amino acids, chromatograms, organic acid analysis, and metabolic screens.

Phlebotomy considerations. The quality of the results is no better than the quality of the specimen collected. The most common sites for phlebotomy of babies are heel sticks and draws from arterial lines. In the NICU, most patients have arterial lines, and typically blood is drawn by the resident, neonatologist, or nurse. Before drawing the specimen, catheters should be cleared of flush solution, in order to avoid possible dilution and/or contamination of the specimen.

Preanalytic concerns in skin puncture. Once the skin is punctured, the blood should flow freely as droplets into the collection tube, and be adequately mixed if anticoagulant is present in the tube. Betadyne contamination increases potassium, phosphorus, chloride, CO_2, and uric acid. Hemolysis frequently occurs, due to poorly performed skin punctures. Hemolysis can cause both method interference (depending on the manufacturer), and a change in observed analyte due to release from the red cells. Higher cellular content and release raises potassium, LD, AST, ALT, CK, and triglyceride. Other analytes like alkaline phosphatase, amylase, and GGT may be decreased due to cellular release of metabolic enzymes.

Heel stick. The standard for practice for heel stick phlebotomy continues to be the NCCLS (CLSI) approved standard. This Standard provides a map for proper placement of the lancet with respect to configuration of the infant's foot.



GUIDELINE 23: Heel sticks.

Phlebotomists should be trained on and follow the NCCLS document, NCCLS Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture: Approved Standard – 4th ed. (1999), H4-A4.
Other key points of this recommendation include the following:

- The optimum depth of the puncture: the selection of the lancet should dictate a puncture depth of < 2.4 mm.
- Avoidance of massage or "milking" the heel is important because interferences from ruptured cellular tissues can be introduced in this manner. There can be a bias in results between skin puncture and venipuncture of approximately 10% higher with skin puncture for some analytes.
- Frequent puncturing of the heels of infants in the NICU can cause edema. This in turn can contaminate tissue fluid, leading to an increase in certain analytes, particularly hemoglobin, potassium, and lactate dehydrogenase.

Other preanalytical factors that may influence results. Among these are the following:

- Prolonged crying during collection may be associated with an increased glucose and lactate.
- Plasma is preferred over serum, providing a better yield with less risk of hemolysis and lysis of platelets.
- Evaporation and transport time should be minimized as much as possible. If centrifugation is necessary, the tubes should be capped, as Na, K, CO_2, and Cl can increase by as much as 30% if spun without caps (140).

Capillary blood differences. TSH, TBG, and T4 are higher in capillary blood than in venous blood. Glucose can be about 0.5 mmol/L higher than plasma, and 0.4 mmol/L higher than whole blood. Due to tissue metabolism, the pH is higher in capillary blood than in venous blood. Capillary tube blood for blood gases must be mixed, sealed, and placed in ice water, as pH can decrease by 0.005 every 10 minutes at room temperature. In order to achieve the turn-around time needed, analysis should be either at the bedside, or in the NICU.

Filter paper specimens for newborn screens should have completely filled circles, or falsely low results may occur.

Method-dependent interferences. It is important to know the degree of interference from high bilirubin, hemoglobin, and lipids. In particular, the lipids derived from total parental nutrition (TPN, intralipids) can affect a variety of analytes. Hemolysis interference on bilirubin is method dependent. The Jendrassik-Grof bilirubin procedure exhibits decreases in concentration due to hemolysis. Conversely, an increase in bilirubin is observed with the 2,5-dichlorophenyldiazonium detergent procedure.

GUIDELINE 24: Preanalytical factors.

- Manufacturers should provide information concerning the impact of preanalytical factors with respect to specific tests.
- Laboratories should consider appending this information to reports.

Specimen labeling. The short blood collection containers that are frequently used, such as the bullet tubes and the microspecimen containers, are often difficult to bar code. In addition, they may not be suitable for some automation devices, preanalytical automation, or total lab automation systems. Such specimens need to be handled off-line. The increased prevalence of multiple births sometimes presents an identification issue in nurseries, with babies yet to be named (e.g., Smith – Twin A, Smith – Twin B) It is important for the laboratory and nursery to agree on a naming convention for multiples that is compatible with the laboratory information system.

Specimen volume. It is crucial to pay attention to the volume of blood taken from a neonate. Hematocrits in newborns and neonates are frequently more than 60%, resulting in smaller yields of plasma and/or serum. The blood volume of neonates can be estimated with nomograms factoring in age and size, to help assess how much blood volume it is safe to take at any one time. Frequent blood draws on premature infants create the risk for iatrogenic anemia, and it is estimated that 64% of babies weighing < 1500 g receive transfusions due to excessive blood draws.

That also puts the infant at risk for issues arising from blood transfusions. Many nurseries use the following rule of thumb: transfusion may be required when ≥ 10% blood volume is withdrawn in 2–3 days. That represents about 80 mL/kg of body weight for a full term and 100 mL/kg for a preterm infant. In recent years, transfusions have decreased due to more transcutaneous monitoring and new instrumentation requiring less blood. And with more *in vivo* monitoring and point-of-care testing, the need for transfusion and the requirement for excessive blood draws are predicted to decrease.

Dead volume. This is defined as the volume of specimen that cannot be sampled from the cup or sample container. Today, most instrument vendors can achieve the precise pipetting of very small sample sizes and offer appropriate containers with small dead volumes of 40–50 µL.

GUIDELINE 25: Sample containers.

Laboratories should use sample containers that are capable of achieving 40–50 µL dead volume.

Urine specimens. It is preferable to use random specimens or timed collections (rather than 24-hour collections) when urine specimens are necessary. Since it is extremely difficult to obtain a complete 24-hour specimen from a non-catheterized infant, the preferred specimen would be from catheterization.

GUIDELINE 26: Urine specimens.

The laboratory should work with clinicians to ensure a proper urine specimen, if needed.

STAT and urgent specimens: turn-around times for results. Turn-around time (TAT) is defined as the time interval from specimen collection to receipt of results. For most pediatric laboratories, it is not uncommon to see requirements for stat turn-around times on 50–60% of the specimens received, compared to 30–40% in an adult setting. A test that is performed off-site, even when performed on a device that performs the test in 10 minutes, can have a TAT as long as

a 30-minute test when performed in the lab or at bedside. In critically ill infants, analytes like electrolytes, blood glucose, and blood gases should have a within-minutes TAT, with everything else ASAP. Critical tests should be available 24 hours a day. For non-critical tests, daily measurements should be adequate.

GUIDELINE 27: STAT testing and TAT.

The laboratory should work with clinicians to ensure appropriate TAT for STAT and non-STAT requests and define the parameters around TAT expectations (e.g., collect to receipt, receipt to verification of results, etc.).

Analytical range. To avoid delays stemming form off-line dilutions, the range of linear response of certain analytes may need to be greater than required in an adult setting. Bilirubin is a good example, where the linear range should extend to 25 mg/dL (428 µmol/L) without the need for dilution.

Fluid and electrolytes in neonates. During the first week of life, small changes in water and electrolyte intake or loss can produce proportionally large changes in total body water and electrolytic content. The preterm infant is more vulnerable to losses through its more permeable skin. This leads to dehydration and to abnormally higher electrolytes. The extracellular water loss may lead to weight loss of 5–10% in a full-term infant, and to as much as 10–20% in a preterm infant. Close monitoring of electrolytes is required. Avoid reporting potassium on visibly hemolyzed specimens, and confirm critical electrolyte results using a specimen obtained from a non-skin puncture, i.e., a venipuncture, or preferably from a line draw.

GUIDELINE 28: Electrolyte monitoring.

- Avoid reporting potassium on visibly hemolyzed specimens.
- Confirm critical electrolyte results using a specimen obtained from a non-skin puncture, i.e., venipuncture, or preferably line draw.

Neonatal Cardiac and Respiratory Function

Oxygen delivery to the tissues depends upon the oxygen-carrying capacity and oxygen saturation of hemoglobin, and on cardiac and respiratory function. Hypoxia is associated with pulmonary hypertension, decreased pulmonary blood flow, acidosis, and organ damage and may be caused by low cardiac output, congenital heart disease, lung disease, anemia, or hemoglobin variants. Hyperoxia, which may occur with oxygen administration in a preterm neonate, is associated with an increased incidence of retinopathy of prematurity and other forms of oxygen toxicity. The therapeutic goal is adequate delivery of oxygen without undue stress on the organs, such as the lungs and retina.

Oxygenation, alveolar ventilation, and acid-base status must be monitored during the neonatal period when cardiac and/or respiratory dysfunctions occur. This monitoring can be performed at the bedside and in the laboratory. Arterial blood gases (ABG) measurements are necessary in the diagnosis of hypoxia and hyperoxia. Continuous non-invasive monitoring of oxygen saturation of hemoglobin by pulse oximetry is a useful tool for oxygen monitoring in the NICU. Interpret ABG values with caution in patients with hyperbilirubinernia, anemia, or in those receiving hyperalimentation, as ABG results may not correlate with pulse oximetry.

Pulse oximetry measures oxygen saturation (sO_2[a]), and transcutaneous oxygen monitors measure the partial pressure of arterial oxygen (pO_2[a]). Though each has limitations, these non-invasive devices monitor trends in oxygenation and are easy to use. The frequency of validation of quantitative ABG measurement depends on the clinical situation of the infant. Values of sO_2(a) obtained by pulse oximetry should be validated by direct CO-oximetry from an indwelling arterial catheter. Blood gas measurements should be performed every six hours for

stable infants and more frequently for critically ill infants. Fetal hemoglobin (HbF) is present in newborns for about six months; it has a higher affinity with oxygen and saturates at a lower pO_2 than HbA. For monitoring O_2 saturation, pulse oximetry is less susceptible to this shift than CO-oximetry.

Newer technologies for measuring blood gases include continuous *in vivo* and *ex vivo* monitoring systems. *In vivo* monitors for blood gases require placement of a sensor/detector in the patient's radial artery, while *ex vivo* monitors draw blood through a catheter, perform measurements externally, and return the blood to the patient. These systems allow for continuous or frequent monitoring without blood loss.

The balance between metabolic carbon dioxide (CO_2) production and ventilatory CO_2 excretion can be estimated by measuring the partial pressure of carbon dioxide (pCO_2) in arterial blood. Management of an increased pCO_2 may involve decreasing CO_2 production (e.g., through sedation or reduction of thermal stress) or by increasing ventilation (e.g., increasing the ventilator rate or tidal volume, reducing airway resistance, administering surfactant). Direct pCO_2 can be measured by ABG or by non-invasive monitors using transcutaneous CO_2 ($tcPCO_2$) or end tidal CO_2 (PCO_2[ET]) monitoring. Though the $tcPCO_2$ method is preferred for preterm neonates, each device has limitations that require validation by ABG measurements.

Preanalytical Concerns for Acid-Base Status

Specimens are obtained from arterial puncture, skin puncture (heel or finger), or from an indwelling catheter placed in the aorta via the umbilical artery or a peripheral artery. Blood obtained from indwelling catheters yields the most accurate measurement of PO_2(a); however, there are risks associated with thrombosis and infections. Indwelling catheters should be flushed and a few drops of blood discarded before collecting the specimen. The radial artery is the usual site for performing an arterial puncture; however, these are hurtful to the baby and cause crying, leading to changes in pO_2(a).

The amount and type of heparin used to anticoagulate the blood must be considered. For example, increased amounts of heparin solution dilute the blood and falsely decrease pCO_2 and bicarbonate. Electrolytes measured on the same sample as ABG can yield falsely elevated sodium or potassium, if sodium heparin and potassium heparin are used. Dry lithium heparin is recommended to avoid dilution effects. Skin puncture, or capillary blood, is obtained from the heel or, less frequently, the finger. Reliable results come from optimizing techniques for obtaining the specimen, adequate perfusion, avoidance of air bubbles, and dilution from anticoagulant. Capillary pO_2 measurements are unreliable in ill infants and not recommended.

The volume of specimen required for blood gas measurements varies from 45 µL to 400 µL, depending on the number of analytes being measured (e.g., blood gases, electrolytes, etc.) and the instrument selected. Although a specimen is considered stable up to 15 minutes for blood gas measurements, the preferred protocol is a specimen collected in a plastic syringe, not placed on ice, and analyzed within 10 minutes. All parameters for ABG (measured and calculated) should be reported, including, PO_2, PCO_2, pH, calculated bicarbonate, and calculated base deficit/excess. Effective communication between the laboratory and the NICU is essential for establishing mutually acceptable turn-around times and appropriate age-related reference intervals.

Neonatal jaundice. Up to 60% of full-term infants and as many as 80% of preterm infants exhibit this condition in the first week of life. Neonatal jaundice is the visual product of bilirubin deposits in the skin and mucous membranes. Physiologic jaundice is defined as 13 mg/dL, or 222 mmol/L (SI units), in the first week of life.

For the routine management of the newborn, the measurement of bilirubin is so common that most newborns receive at least one bilirubin measurement. Total bilirubin is generally used as the initial indicator of jaundice. Accurate bilirubin measurements are vital in the assessment and therapeutic monitoring of neonatal jaundice and in providing a differential diagnosis for hepatic immaturity vs. the more life-threatening consequences of Rh-antibody-induced hemolytic jaundice. Most cases of elevated bilirubin are due to immature hepatic function impacting the conjugation

of bilirubin. Total bilirubin measurements are important in the detection of hemolytic jaundice. Since only conjugated bilirubin crosses the blood/brain barrier, direct or conjugated bilirubin measurements and fractionation may be useful in diagnosing hepatic disorders, hemolysis, hereditary disorders of bilirubin metabolism, and in the prevention of brain injury or kernicterus and its associated spasticity, hearing loss, and mental retardation.

Causes of bilirubin overload include increased production of bilirubin, increased ratio of red blood cell to body weight as compared to adults, a shorter red blood cell lifespan, hepatic immaturity causing decreased conjugation, and decreased hepatic clearance. Bilirubin toxicity in the neonate can occur from impaired albumin binding (either insufficient binding sites, or low albumin levels) that can increase bilirubin levels. Both acidosis and drugs that displace bilirubin from albumin increase bilirubin concentrations. Recent studies in neonatal therapy for anti-immunoglobulin positive infants suggest that treatment with intravenous immunoglobulin is effective as an alternative therapy for isoimmune hemolytic jaundice and can reduce the need for exchange transfusion (*141*). However, further well-designed studies are needed before routine use of intravenous immunoglobulin can be recommended for the treatment of isoimmune hemolytic jaundice.

GUIDELINE 29: Testing for liver function.

Liver function should be evaluated using a combination of bilirubin testing and liver function enzyme testing.

Glucose in neonates. Both high and low concentrations of glucose can be dangerous to the neonate. Neonates are at risk of hypoglycemia immediately after birth due to increased glycolytic enzyme activity, with the risk increased in preterm neonates with low hepatic glycogen stores. Hyperglycemia may occur following glucose administration, particularly in the preterm infant, due to a sluggish insulin response. Management of glucose in the at-risk groups is essential. For moderately preterm or growth-retarded infants, glucose should be monitored with breast feedings and with formula feedings. For infants with acute illness, fluid management has to be fairly aggressive, and monitoring blood glucose levels is key. In unexpected hypoglycemia, the infant should be evaluated for inborn errors of metabolism. Frequent monitoring is often performed using point-of care (POC) glucose monitors. Because of the expected higher hematocrit levels in neonates in general, and in neonates receiving oxygen therapy in particular, devices and test strips must be evaluated and correlated to laboratory methods for appropriate interpretation of results. In addition, whole-blood POC glucose is approximately 11% higher than serum or plasma values.

Although there is no uniform agreement for the cutoff value for hypoglycemia, critical glucose results (generally < 40 mg/dL, 2.2 mmol/L) obtained by POC devices should be confirmed by the laboratory. Many NICUs try to maintain concentrations between 3 mmol/L (> 54 mg/dL) and 10 mmol/L (< 180 mg/dL) (*142,143*).

GUIDELINE 30: Critical glucose measurements using POCT devices.

Glucose measurements of < 2.22 mmol/L that are performed on POCT devices should be confirmed by the laboratory.

Creatinine in the first few days of life reflects maternal function. Interpretation of creatinine results is complicated by rapid changes in extracellular volume and glomerular filtration rate. Changes in creatinine vary with gestational age, and the absence of an expected drop may indicate compromised renal function.

Lactate can accumulate in tissues, blood, and cerebrospinal fluid (CSF) from anaerobic metabolism often caused by crying. Lactose measurements indicate adequacy of recent or current oxygen delivery to the tissues, and they can be essential in the diagnosis of inborn errors of metabolism. Small point-of-care systems for whole blood lactates are now available for NICU or bedside settings.

Calcium and phosphorus are incorporated into the bone matrix during the last trimester. Therefore, the preterm infant has greater needs for these two minerals than term infants. In parenteral nutrition (PN) solutions, the interaction

of calcium and phosphate is complex and influenced by many factors. Calcium and phosphorus requirements many exceed the solubility of these two minerals and lead to precipitation and embolization, or catheter occlusion. Optimal delivery is restricted by the pH of the solution that in turn is determined primarily by the amino acid concentration of the PN solution. Therefore, preterm infants on long-term PN are also at greater risk of developing osteopenia of prematurity (metabolic bone disease) and subsequent fractures. Routine lab monitoring of calcium, phosphorus, and alkaline phosphatase will identify those patients most at risk. In metabolic bone disease, alkaline phosphatase levels are elevated; serum calcium may be normal at the expense of bone loss, and the phosphorus level is low. Urinary phosphorus is low due to renal tubular reabsorption of phosphorus and urinary calcium is elevated. Serum 25-hydroxyvitamin D levels may also be measured, though pediatric multivitamin preparations provide adequate amounts of this vitamin to maintain normal serum levels and prevent both toxicity and deficiency.

Calcium rises in the first hours of life following a parathyroid hormone response, and drops in the next 24–48 hours. Total calcium underestimates physiologically active calcium (ionized calcium), if the serum albumin and/or pH are low. Therefore, ionized calcium is the preferred measurement when an accurate assessment is needed, particularly in hypocalcemia of the preterm infant.

GUIDELINE 31: Calcium testing in the neonate.

Ionized calcium is the preferred method for testing of calcium.

Therapeutic drugs. Approximately 12% of all drugs prescribed in the U.S. are for children younger than nine years of age. For premature infants < 1000 g, the number of drugs given during hospitalization averages 15–20. Therefore, aggressive monitoring is necessary to prevent toxicity in the smallest patients, as pharmacokinetics are significantly different in babies. Absorption is altered in the newborn period due to gastric pH and emptying time. There is a volume of distribution (VD) difference due to body composition, fat content, and water content. Clearance is slower in premature infants due to immature hepatic and renal function. Due to the immaturity of enzymatic pathways and decreased protein binding, biotransformation of the drugs into metabolites and the bio-usable form is lower.

Testing for Congenital or Infectious Disease

Group B Streptococcus. Group B streptococcus (GBS) is present in the vagina and gastrointestinal areas of 10–30% of healthy women, though it rarely causes an infection. Each year infections develop in more than 50,000 pregnancies. These infections may be present in the uterus, amniotic fluid, urinary tract, and incision sites, e.g., cesarean section. During birth, the baby may become infected by inhalation or ingestion of the bacteria. Approximately 8,000 babies in the U.S. contract serious GBS disease each year, with a 10% fatality rate. Up to 20% of the babies who survive GBS-related meningitis are left permanently handicapped (*144*).

In newborns, GBS is the most common cause of sepsis and meningitis and is a frequent cause of newborn pneumonia. GBS disease is more common than other, better known, newborn problems such as rubella, congenital syphilis, and spina bifida. Long-term medical problems in survivors, particularly in those who developed meningitis, may include hearing or vision loss, and varying degrees of physical and learning disabilities, including cerebral palsy.

Infected infants may display symptoms as early as six hours or as late as two months following birth.

Early diagnosis and initiation of antibiotic therapy in the neonate is often delayed due to the nonspecific, subtle, and often mild clinical signs and symptoms. Delays in treatment are associated with significant neonatal mortality and morbidity due to rapid progression and severity of infection in the newborn. The time frame required for definitive microbiologic evaluation is too long to withhold antibiotic therapy; furthermore, multiple cultures may be required for pathogen recovery. Cultures can also be contaminated, making interpretation difficult. Initiation of antibiotic therapy is often based on clinical impression.

In 1996, the Centers for Disease Control, the American College of Obstetrics and Gynecology, and the American

Academy of Pediatrics CDC, in collaboration with the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics, issued aggressive guidelines for prenatal screening and prevention of GBS disease. These guidelines were revised in 2002. Newer studies showed that routine screening for GBS prevents more cases of early-onset disease than the risk-based approach. This data supported the conclusion that all pregnant women should have vaginal and rectal GBS screening cultures at 35–37 weeks' gestation (*144*). Recommendations included advice that laboratories adopt optimal screening practices to identify GBS and to promptly report test results so that GBS-colonized pregnant women can receive antibiotics during labor (*145*).

GUIDELINE 32: GBS screening for pregnant women.

Follow the CDC guidelines on perinatal screening for GBS on all pregnant women from 35–37 weeks of gestation.

Efforts to identify sensitive and reliable biomarkers have frustrated decades of investigators. For example, there are no uniformly accepted hematological criteria that effectively distinguish infected from non-infected infants. The search for a reliable early laboratory indicator for neonatal sepsis is further fueled by the recent rise of antibiotic resistance in pathogenic bacteria associated with indiscriminant use of antibiotics, disruption of infant-maternal bonding related to early hospital release, subjection of newborns to intravenous therapy, and the drive for medical cost containment.

The acute phase proteins (fibrinogen, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, C-reactive protein, and alpha-1-acid glycoprotein) have been the subject of numerous investigations (*146,147*). The lag time between onset of infection and production of acute-phase proteins explains the disappointing sensitivity and positive predictive values for testing. Recent studies suggests that a combination of CRP, interleukin-6, and procalcitonin testing in the early postnatal period may detect infection in a higher-risk, asymptomatic infant with an infected mother (*147*). Vaccine development is still underway as the ultimate prevention.

Point-of-Care Testing (POCT)

The small specimen requirements and the rapid turn-around time make point-of-care very well suited for the neonate. Many POCT devices are capable of performing multiple analytes on a whole blood specimen of 100 µL or less. This is less than the amount required to be drawn, sent to the lab, spun down, and aspirated into the analytical device.

It is necessary to validate these point-of-care devices for neonates with respect to typical interferences and to the differing range of concentrations that are observed in adults (e.g., lipemia from TPN, high hematocrits in newborns). For glucose, it is important to use a system that is reliable in the low glucose ranges of 2.22 mmol/L or less. It is often difficult for manufacturers to validate every circumstance due to the difficulties in obtaining representative samples.

Both *in vivo* and *ex vivo* monitors for blood gases and electrolytes are available and applicable to the neonatal population. The "ideal" point-of-care device is small, robust, lightweight; it uses a small sample size and is easily transported. Point-of-care devices fall in two groups: electronic-based point-of-care devices and non-electronic-based testing. The non-electronic devices have been in use for many years, include manual procedures, are indicator based, and generally produce qualitative or semi-quantitative positive/negative results. Examples applicable to neonates include urine dipsticks and pregnancy tests. The electronic devices are handheld formats, and can be transportable from patient to patient or be stationary, as in the NICU. Methods applicable for point-of-care include electrochemistry, reflectance photometry, and immunology-based methods.

Examples of POC analyzers currently used in the NICU setting include the SureStep Probe for glucose from LifeScan, the Hemocue for hemoglobin from ITC, the Hemochron Junior Signature for ACTs (activated clotting times), and the I-Stat for blood gases, electrolytes, and creatinine.

Differences in results. The result from a blood glucose meter may not be the same as that from laboratory for a number of reasons. Glycolysis during transport can lower the laboratory result (as compared to the bedside result). Glucose in whole blood can be as much as 11% higher than glucose in plasma due to spin time and contact with red cells. At extreme hematrocrits, predicted whole blood glucose does not correspond with the true whole blood glucose. With a device that lyses cells, this is not as much of an issue.

Other observed differences may be due to different calibration schemes as well as sample matrix effects. Point-of-care devices may be less precise than the laboratory devices. In the lab, CVs for glucose are typically ≤ 5%. FDA approval typically requires 20%. Preanalytic issues, as well as staff compliance and competency in performing the point-of-care tests, can also contribute to the differences.

A recent proficiency survey (Proficiency Survey AAB 2nd Q 2001) compared different glucose meters and demonstrated a wide spread in recovered values for each sample (Table VII-2).

Table VII-2. Comparison of Glucometers: Recovered Values

Proficiency Survey* (AAB 2nd Q 2001)	Sample 1	Sample 2	Sample 3
Bayer Glucometer	153.7	76.1	34.1
HemoCue	284.8	155.4	68.3
Lifescan OT II1	48.8	91.7	59.0
Lifescan SureStep	190.6	116.7	74.7
Medisense PCx	164.6	96.3	55.2
Roche Advantage	154.7	88.6	45.6

*mg/dL

Suggestions for avoiding potential errors in using blood glucose meters include the following (*143*):
- Know the limits of glucose meter and test strip measurements.
- Know if measurements reflect plasma (conversion) or whole blood.
- Understand changes in blood composition in critically ill patients.
- Use O_2-insensitive test strips in patients undergoing O_2 ventilation.

New POCT technologies are emerging rapidly. With continuous *in vivo* and *ex vivo* monitoring devices, and an increasing number of minimally invasive devices as well as non-invasive devices, many are particularly well suited to the pediatric population. Some examples are given here.

An example of an *in vivo* application, or *ex vivo* monitor with an *in vivo* line, is the case where blood from an arterial line passes into an *ex vivo* monitor for blood gases and limited electrolytes (pH, pCO_2, pO_2, sodium, potassium, hematocrit) and then re-enters the infant circulation. Minimal blood loss confers a big advantage for this application of continuous monitoring. Recent studies demonstrate good agreement with laboratory analyzers (*148,149*).

The BiliCheck point-of-care bilirubin device from SpectRx, Inc., is a non-invasive handheld device using multi-wavelength spectral analysis to take a transcutaneous measurement on the baby's forehead. A recent study (*150*) of 490 pre-discharge term and near-term racially diverse newborns showed good agreement vs. a gold standard HPLC measurement (r = 0.91, range 0.2–18.2). In addition, skin color was not found to be a significant variable, and infants potentially at high risk for developing hyperbilirubinemia after 48 hours were able to be identified before being sent home.

The Cygnus GlucoWatch by Biographer is a minimally invasive device for monitoring glucose. Glucose is extracted through the skin by reverse iontophoresis using an applied electrical potential, and detected by electrochemical enzymatic sensor. Three measurements per hour can be obtained.

Orthogonal polarization spectroscopy (OPS) uses sublingual in vivo imaging to measure red blood cells and a partial CBC. A probe placed under the tongue measures the flow rate of blood through the tongue's small capillaries.

GUIDELINE 33: Point-of-care testing for neonates.

- The laboratory should consider using non-invasive point-of-care testing as an alternative to laboratory testing to minimize blood draws. Small specimen requirements and rapid turn-around time make point-of-care applications well-suited for neonatal patients.
- Neonatal blood differs from adult blood. Therefore the laboratory should validate the effect of interferences (e.g., high hematocrits) and differing concentration of analyses from neonates vs. those from adults on the same POCT devices.

Blood Typing and Direct Antiglobulin Test (Direct Coombs)

These tests are appropriate in neonates in the following situations:
• When the mother has group O blood type.
• When the mother has Rh-negative blood type.
• When an antibody screen indicates that the mother has an antibody that could harm the baby.
• When the baby has clinical symptoms that might be explained by the results of these tests.

There are two main reasons to perform blood typing of a newborn The first is to determine whether the mother is a candidate for receiving Rh immunoglobulin post-delivery, in order to prevent the development of maternal Rh-antibodies that could harm the developing fetus in future pregnancies. Only Rh-negative mothers of Rh-positive infants would receive the treatment. The second reason is to identify the neonates at risk of developing hemolytic anemia. In that case, babies with either group A or B blood type may react to antibodies produced by mothers with group O blood type. The direct antiglobulin test can determine if maternal antibodies are reacting with the baby's blood cells. A positive test means the baby is at risk of developing hemolytic anemia, and a negative test indicates that the mother's antibodies are not reacting with the baby's blood, so usually the infant is not at risk. Many reactions to maternal antibodies are self-correcting and produce only mild symptoms. A hemoglobin test on the infant can gauge the extent of anemia.

The primary limitation of the direct antiglobulin test is false negatives. The presence of maternal antibodies in the baby's blood may be below the threshold for detection. Thus a negative direct antiglobulin test result does not rule out the possibility of anemia. Conversely, a positive result does not necessarily mean that the baby will develop anemia.

GUIDELINE 34: Critical testing for neonates prior to release from the hospital.

The following critical tests should be performed before the infant leaves the hospital:

Rubella (German measles) immunity	Hemoglobin abnormalities screen, based on family/medical history
HIV	Newborn screening testing mandated by the governing body
Hepatitis B screen	Toxoplasmosis
Hemoglobin (CBC) and hematocrit	RH antibody screen

SECTION VIII.

Newborn Metabolic Screening

Each year four million infants in the U.S. are screened to detect conditions that threaten their lives and long-term health (*151*). Testing performed at birth serves to detect an infant with a metabolic disorder, assess the likelihood of anemia, detect abnormal genes, and, if applicable, determine the maternal need for Rh immune globulin. Newborn screening for metabolic disorders is mandated in all of the United States, and in its territories and possessions. Each state is responsible for determining which tests should be performed on newborns; however, healthcare providers may choose to perform additional testing.

Title XXVI of Children's Health Act 2000 was passed to provide national guidance and standardization in order to expand newborn and child screening programs for Screening for Heritable Disorders in Newborns and Children (*152*). The implementation involves four agencies: Health Resources and Services Administration (HRSA); Agency for Healthcare Research and Quality (AHRQ); the Centers for Disease Control and Prevention (CDC); and the National Institutes of Health (NIH). Worldwide, the Association of Public Health Laboratories comprises more than 250 laboratories in the United States and 45 other countries, partnering with the CDC to provide services aimed at ensuring the quality of testing. These services include filter paper evaluation, training, consultations, and proficiency testing (*152*).

Metabolic Screening Conditions and Testing

Metabolic deficiencies cause symptoms that range in severity. At present, all states require screening for phenylketonuria (PKU) and congenital hypothyroidism (inactive thyroid gland), which lead to mental retardation when untreated. In 2001, all states but Washington required testing for galactosemia, and all but three states offered a screen for the hemoglobinopathy causing sickle cell disease (*151*). Other metabolic screening tests are available and may be performed based on state requirement or family history, or to diagnose a symptomatic infant. State testing is typically performed by elution of dried blood spots from standardized filter paper cards (Guthrie cards) prepared within a few hours of birth by heel stick blood collection (*153–155*).

Table VIII-1. Number of States (including the District of Columbia) That Screen for Metabolic Conditions (151)

Congenital hypothyroidism	All 51
Phenylketonuria (PKU)	All 51
Galactosemia	All 51
Hemoglobinopathies	All but 3 (44)
Biotinidase deficiency	21
Homocystinuria (HCU)	18
Maple syrup urine disease (MSUD)	24
Cystic fibrosis (CF)	6
Congenital adrenal hyperplasia	18
Toxoplasmosis	2
HIV	1

An example of the efficacy of testing is demonstrated by statistics from California in the period from 1980 through 1997 (*156*). More than 8.5 million infants were tested in the newborn screening program. This testing detected 2,664

cases of primary congenital hypothyroidism, 320 cases of classical phenylketonuria (1 in 12,000), and 116 cases of transferase deficiency galactosemia. Since initiating screening for hemoglobin disorders, more than 4 million infants have been tested. From 1995 through 1997, nearly 1,000 cases of sickle cell disease were identified and 131 cases of clinically significant hemoglobinopathies were referred for follow-up care. In 1997, the California State Legislature passed Senate Bill 537, mandating the addition of 17 disorders to the current program. The list includes cystic fibrosis, congenital adrenal hyperplasia, biotinidase deficiency, as well as a variety of aminoacidopathies and fatty acid oxidation disorders.

Screening and Confirmatory Tests

State testing involves both screening and confirmatory testing. Some testing may be outsourced to state-sanctioned contract laboratories. Screening laboratories ascertain the possible presence of a birth defect or congenital disorder. When a screening test result is positive, the patient is referred for a definitive clinical evaluation that includes diagnostic testing at a confirmatory laboratory. Confirmatory laboratories perform a battery of diagnostic tests to help determine if a birth defect or congenital disorder is actually present (*152–156*).

Methodologies

Screening tests are performed on dried-blood-spot specimens collected from newborns. Depending on the particular test, various standard and state-of-the-art methods are used including colorimetric, immunoassay, radioimmunoassay, HPLC, fluorometric, PCR, or alternate DNA analysis. One technique that is rapidly gaining acceptance is tandem mass spectrometry (MS/MS), which can detect up to 30 specific diseases. A few hospitals offer this test to all parents, but, in most cases, the parents must request that this extensive, but relatively inexpensive, screening be performed.

Congressional Interest

In the U.S., a congressional committee was convened to provide national oversight into the issues surrounding newborn screening. In its report, the Committee urged the availability and accessibility of newborn screening services to apply public health recommendations for expansion of effective strategies. HRSA, in collaboration with the CDC and the NIH, was encouraged to implement a strategy for evaluating and expanding newborn screening programs, pilot demonstration projects, and the use of contemporary public health recommendations on specific conditions, such as cystic fibrosis and fragile X syndrome. The Committee further directed that "tangible steps be taken to protect patient privacy and to avert discrimination based upon information derived from screenings."

A Newborn Screening Task Force was convened by the American Academy of Pediatrics (AAP) and funded by Maternal and Child Health Bureau, Health Resources and Services Administration (MCHB, HRSA). The summary of recommendations from the American Academy of Pediatrics (AAP) Task Force included the following (*154*):

- Use a systems approach—not just testing.
- Follow accepted guidelines.
- Coordinate and integrate programs and data.
- Pilot new tests.
- Monitor performance and evaluate program.
- Involve and inform parents.
- Convene a statewide advisory group.
- Safeguard blood samples.
- Provide adequate financing for testing, diagnosis, and treatment.

Testing for Specific Conditions Detectable in the Newborn

Congenital hypothyroidism (*154,156,157*)**.** Congenital hypothyroidism occurs due to a malfunction of thyroid gland development (either complete absence [aplasia], partial glandular development [hypoplasia], or an ectopic location) resulting in insufficient production of thyroxine, the primary growth-regulating hormone necessary for proper nervous system development. The absence of this hormone causes slow growth and mental retardation.

Mental retardation can be avoided if detection and treatment with thyroid supplements occur within a few days of birth. Untreated congenital hypothyroidism is the most common cause of mental retardation, affecting nearly 500 infants per year in the U.S.

Testing performed. Screening consists of measuring either total thyroxine (T4) or thyroid-stimulating hormone (TSH) or both. The cutoff point for total T4 varies, depending on the program and the manufacturers' imprecision variability at the low concentrations needed for detection of presumptive positive specimens in eluted dried blood spots.

Limitations of congenital hypothyroidism screen. Thyroid testing measures the amount of hormone that is present when the blood is taken. At birth, thyroid hormones from the mother are present in the baby's circulation. The presence of the mother's thyroid hormones can mask the baby's low thyroid hormone level. Discharging a baby shortly after delivery does not allow enough time for the mother's thyroid hormones to disappear from the baby's circulation. To more accurately diagnose congenital hypothyroidism, it is recommended that the specimen is collected between two and six days of age. The vast majority of infants with congenital hypothyroidism are detected on the first specimen, but physicians should remain alert to developing clinical symptoms in spite of a normal initial screen. The most significant cause of a false initial positive result for primary congenital hypothyroidism is specimens collected from infants who are less than 24 hours old. Recent improvements in assay formulation seem to have significantly reduced these false initial positive results.

If the baby is discharged prior to 48 hours of age, thyroid testing should be performed as close to the time of discharge as possible, but no later than seven days of age. If the baby's blood was collected before it was 12 hours old, a second specimen should be tested before two weeks of age.

Guideline 35: Thyroid testing for newborns.

Manufacturers should provide thyroid assays that are compatible with the testing of eluted dried blood spots.

Premature infants. In some premature infants a transient physiological effect due to immaturity of the pituitary hypothalamic axis results in lower TT4 results with concomitant elevated TSH. Such observations require close monitoring to ensure that the T4/TSH levels approach normal values as the infant matures.

Phenylketonuria (PKU). PKU is the most common genetic abnormality in the USA with 1 in 50 individuals carrying the gene, and with 1 in 15,000 babies testing positive. PKU is an autosomal recessive deficiency of the enzyme phenylalanine hydrolase, preventing the conversion of the essential amino acid phenylalanine into tyrosine, using tetrahydrobiopterin as a cofactor. Normal metabolism of phenylalanine results in a serum concentration between 30 µM and 180 µM (0.5–3 mg/dL). When affected individuals eat foods high in protein such as milk (including infant formula), meat, eggs, and cheese, phenylalanine will accumulate in the blood, urine, and central nervous system. Phenylalanine is abundant in these high-protein foods and is the predominant component of the artificial sweetener, aspartame. Inheritance of PKU causes developmental delays, seizures, acid odor, and severe mental retardation, if not detected and treated early. Restricting the diet with respect to phenylalanine and monitoring serum levels have proven effective in treating this condition if initiated as soon as possible and before four weeks of age. This treatment must continue throughout the patient's life (*154*).

Maternal PKU and hyperphenylalaninia. With the advent of screening programs within the last 40 years, more women with homozygotic expressed PKU have reached childbearing age. Poorly controlled PKU in such women can lead to an increased risk of miscarriage; more than 90% of their offspring exhibit intrauterine growth retardation, microcephaly, mental retardation, and/or primary congenital heart defects. These infants show a transient rise in PKU values, which fall to normal within 24 hours of birth. PKU mothers should maintain levels of phenylalanine between 120 and 360 µM in order to avoid damaging the developing fetus.

Limitation of PKU test. Collecting an insufficient amount of specimen will affect the test result. Specimens for testing should be collected from infants older than 24 hours and younger than 7 days. Screening prior to 24 hours of age may yield an inaccurate result. Causes of false initial positives for PKU include prematurity and parenteral feedings.

Cystic fibrosis. Cystic fibrosis is an autosomal recessive disorder characterized by dysfunction of several exocrine systems. The incidence of cystic fibrosis is 1 in 2,500 Caucasian infants; it is somewhat lower among other ethnic groups (*154*).

The initial presentation may be in the neonatal period with meconium ileus or later in infancy or childhood with growth problems, malabsorption and malnutrition, and/or pulmonary disease. Severity of symptoms is variable. Death usually occurs between the second and fourth decades of life as a result of obstructive pulmonary disease and infection.

Laboratory testing. Elevation of immunoreactive trypsinogen (IRT) in a dried blood spot is the current screening method for CF. False positives and false negatives are known to occur, with false negatives occurring more frequently in neonates with meconium ileus.

Screening practice considerations (Table VIII-2). Elevations of trypsinogen decline after the first several months of life, so while exact timing of specimen collection in the neonatal period is not critical, the collection of the second screening specimen to follow up an initial abnormal screen should occur no earlier than 21 days to avoid an increased number of false positives, and no later than 60 days to reduce the risk of false negatives. Use of the IRT test in older infants and children is not recommended; a sweat test is advised if CF is suspected in this older group. Sweat testing by personnel trained specifically in an accurate method is essential for proper diagnosis of cystic fibrosis.

Table VIII-2. Examples of Cystic Fibrosis Testing Decision Tree in Three States

Abnormal Results	Likely Causes	Recommended Follow-up
IRT ≥ 90 ng/mL (CO/WY) ≥ 100 ng/mL (MT)	Cystic fibrosis Early collection of specimen False positive	Second newborn screening specimen collected at 21–60 days of age
Repeat IRT ≥ 70 ng/mL(CO/WY) ≥ 80 ng/mL (MT)	Cystic fibrosis Early collection of specimen False positive	Diagnostic sweat testing

Galactosemia. Galactosemia testing is performed in all 50 United States plus the District of Columbia. It is an autosomal recessive disorder with an incidence of 1 in 60,000 to 1 in 80,000 for the most common enzyme deficiency GALT (galactose-1-phosphate uridyl transferase), which prevents the breakdown of galactose to glucose. Other enzyme deficiencies such as galactokinase and/or uridine-diphosphategalactose-4-epimerase are less common. Babies who inherit this disorder cannot metabolize the sugar galactose found in milk, breast milk, formula, and other foods. Within the first two weeks of life, untreated infants born with this condition experience vomiting, liver disease, mental retardation, cataracts, and failure to thrive. *E. coli* sepsis may present and cause fatality if not detected early. Providing a milk-free diet is the recommended treatment for galactosemia, and can improve the outcome.

Testing. Elevated galactose levels may be detected using an *E. coli* microbiology test, but most screening laboratories use a combination of the Buetler fluorescence test for GALT deficiency and/or a fluorometric test for galactose (Hill test).

Limitations of galactosemia screen. The test does not detect carriers. Patients having had transfusions may appear to have adequate levels of enzyme for two to three months, obscuring detection. For galactosemia, the most common cause of false positives has been heat denaturation of the enzyme during transport (*154*).

GUIDELINE 36: Sample handling of specimens.

Laboratories responsible for collection of specimens should ensure proper sample handling and transport conditions to avoid loss of enzyme activity.

Hemoglobinopathies. Infants with sickle cell disease or other hemoglobinopathy are highly susceptible to viral and bacterial infections that markedly increase morbidity and mortality. Neonatal screening for hemoglobinopathy is routine in the United States and many other countries because early diagnosis and treatment (e.g., prophylactic use of penicillin) enhances both survival and long-term outcome (*154*).

Biotinidase deficiency. Biotinidase is an enzyme that liberates the essential cofactor biotin from its bound form so that it can be used by the body. Deficiency of the enzyme in serum results in improper functioning of several other enzyme systems, leading to irreversible neurological damage. This autosomal recessive disorder has an estimated incidence of 1 in 60,000 births (*154*).

Type of test. *A* colorimetric assay for biotinidase is performed on a dried blood spot. Affected infants and children have 0% to 10% of normal adult activity. Levels between 10% and 30% of mean normal activity levels are considered partial biotinidase deficiency.

Timing. Optimal timing for testing is unknown. Enzyme deficiency has been demonstrated in cord blood; therefore, any specimen obtained after birth is anticipated to be adequate. Symptoms have not developed in most patients before two months of age, but one patient was symptomatic at three weeks. Thus, rapid turnaround may be needed. The mean age at onset of symptoms is five to six months.

Stability of specimen. Samples stored for longer than 18 months at room temperature or higher had no detectable activity. Activity was detected in samples less than 18 months old. Samples analyzed 1, 30, and 60 days after collection were stable. Specimens are stable frozen at -70 ˚C for 3 years; samples frozen at higher temperatures (-20 ˚C) may lose activity, which may lead to inappropriate diagnosis of partial deficiency.

Confirmation. Both a colorimetric and a more sensitive radioassay of serum are available to confirm screening results. On the basis of families studied to date, heterozygotes (carriers) can be differentiated from affected and normal individuals with 90–95% accuracy.

Accuracy of screening test. The false negative rate is unknown. Rare (< 1%) false-negative test results may occur with the use of sulfonamides. All samples tested after the newborn period should be checked for the presence of sulfonamides. The false positive rate is unknown.

Ongoing studies. A pilot screening program was initiated at the Medical College of Virginia by Barry Wolf. Screening is also being conducted in 15 countries worldwide. Follow-up of screening cases is in progress. Information is needed concerning incidence, natural history, efficacy of treatment (including evaluation of older, previously asymptomatic patients), parameters for optimal treatment, and heterogeneity of the disorder.

Congenital adrenal hyperplasia. Congenital adrenal hyperplasia (CAH) includes a group of autosomal recessive disorders, each characterized by a deficiency of one of the enzymes needed to transform cholesterol to cortisol (hydrocortisone) (Table VIII-3). These enzymes are 20,22-hydroxylase; 3-hydroxysteroid-dehydrogenase; 17-hydroxylase; 21-hydroxylase; and 11-hydroxylase. The incidence in selected populations varies from about 1 in 10,000 to 1 in 25,000 (*154*).

Table VIII-3. Characteristics of Three Types of Congenital Adrenal Hyperplasia (CAH)

Salt-Losing CAH	Simple Virilizing CAH	Late-Onset CAH
a. No cortisol = hypoglycemia	a. Normal or near normal cortisol	a. Normal cortisol
b. No aldosterone = salt and water loss	b. Increased cortisol precursors (17-hydroxyprogesterone)	b. Normal aldosterone
c. Increased cortisol precursors (17-hydroxyprogesterone) = salt-losing tendency	c. Increased aldosterone to compensate for salt-losing tendency	c. Increased 17-hydroxyprogesterone (moderate)
d. Increased androgens = masculinization	d. Increased androgens = masculinization	d. Increased androgens = masculinization

An affected infant is characterized by hyperfunction and increased size (hyperplasia) of the adrenals, hence the name congenital adrenal hyperplasia. Among the various forms of CAH, the 21-hydroxylase deficiency is the most frequent, representing more than 90% of all cases. The most severe form of 21-hydroxylase deficiency is associated with salt wasting. The inability to synthesize cortisol leads to an increase in ACTH and a build-up of precursors to cortisol (i.e., 17-hydroxyprogesterone and androgens). Aldosterone production is also impaired due to the total absence of 21-hydroxylase. Although there is an increase in both renin and angiotensin, aldosterone production remains low or nonexistent. Non-detection of an affected male infant can lead to early death within the first two weeks of life.

The simple virilizing form of CAH is caused by a partial deficiency of the 21-hydroxylase enzyme. Because this enzyme deficiency is only partial, these subjects are able to produce near normal or normal amounts of cortisol due to increased ACTH output. However, similar to the salt-losing patients, simple-virilizing patients experience an increase in the production of 17-hydroxyprogesterone as well as adrenal androgens. The elevated 17-hydroxyprogesterone produces a salt-losing tendency. Because the 21-hydroxylase deficiency is partial, the adrenals are able to increase production of aldosterone to compensate for salt loss.

In both of these forms of CAH, the increased production of adrenal androgens causes concern. The most important adrenal androgen secreted in large amounts is androstenedione. This steroid is not androgenic by itself. However, approximately 10% of androstenedione is metabolized in the body to testosterone, a potent androgen. Excess androgen production during fetal life, associated with salt-losing and simple-virilizing CAH, masculinizes the external genitalia of female infants, leading to potential misclassification of a female infant as male.

Late-onset CAH refers to a mild deficiency of the 21-hydroxylase, which manifests with excess androgen production in childhood or adolescence. While the partial deficiency allows the compensated production of normal amounts of cortisol and aldosterone, affected individuals produce increased amounts of cortisol precursors (17-hydroxyprogesterone) and adrenal androgens. In both male and female, this results in rapid growth and early virilization. In girls, this can also result in masculinization and abnormal menses.

Type of test. Enzyme immunoassay or radioimmunoassay for measurement of 17-OHP in 21-hydroxylase deficiency can be performed on dried blood spots.

Timing. Elevation of 17-OHP is present at birth, although levels obtained before 24 hours of age may be physiologically high. Rapid turn-around time may be needed to detect boys and those nonvirilized-undetected girls who may present with early onset adrenal crises and salt losing. Premature infants may have false positive test results. Screening in the first 48 hours may increase the false positive rate, but further study is needed. Screening at one to two weeks of age detects some additional cases of simple virilizing CAH and increased numbers of the non-classic form of 21-hydroxylase deficiency.

Stability of specimen. No decomposition of 17-OHP has occurred after periods of as long as 30 days in blood dried on filter paper stored at room temperature.

Confirmation. Quantitative measurement of plasma 17-OHP is available from many commercial laboratories. A relatively small sample of blood is required.

Accuracy of screening test. The false negative rate is low and the screening test detects most cases (95%) of 21-hydroxylase deficiency. With an initial screen of more than 65 ng/mL, 3% of salt wasters may be missed if screened before 24 hours of age.

The false-positive rate ranges from 0.2% to 0.5%, depending on the cutoff level chosen. The cross-reaction of steroid compounds related to 17-OHP depends on the antiserum used in the immunoassays of steroids and whether organic solvent extraction is included in the testing protocol (*154*).

GUIDELINE 37: Quality of testing for newborn screening.

For optimum screening detection and outcome, babies should not be discharged from the hospital before specimens for newborn screening accurately portray the concentrations of the substances being tested under the statutes of the governing body.

SECTION IX.

Advances in Newborn Screening Using MS/MS

Mass spectrometry as an analytical technique has been used for many years in both qualitative and quantitative research applications. Typically the applications for biological compounds involved the use of gas chromatography to separate the compounds of interest, prior to injection into and analysis by the mass spectrometer. GC/MS is typically a slow process that does not lend itself well to mass screening applications. With the development of tandem mass spectrometry (MS/MS), these difficulties were overcome and the specialty analysis that was both fast and sensitive became available. This analysis was initially used for specialized clinical testing to measure carnitine esters in the blood and urine of children suspected of inborn errors of metabolism (*158*).

Mass spectrometry separates and measures the mass-to-charge (m/z) ratio of ions that have been produced from fragmentation of parent molecules in the ionization chamber of the mass spectrometer. The most common techniques consist of separating the substances to be measured in a gas chromatograph, followed by fragmentation and measurement in a single mass spectrometer. The tandem mass spectrometer usually consists of a pair of analytical quadrupole mass spectrometers separated by a reaction chamber or collision cell. (In most instruments the collision cell is actually a third quadrupole.)

The substance to be analyzed undergoes a *soft* ionization procedure (e.g., fast atom bombardment or electrospray) to create quasimolecular ions. The substance is then injected into the first quadrupole, which separates the *parent ions* from each other. The ions pass (in order of m/z ratio) into the reaction chamber or collision cell, where they are subjected to controllable fragmentation by collisions with inert gases (like argon or helium). These fragments of the parent ions then pass into the second analytical quadrupole where they are analyzed according to the m/z ratios of the fragments.

Electrospray ionization is a “soft ionization” technique that enables the direct analysis of biological high molecular weight substances such as proteins previously considered non-candidates for mass spectrometry. Compounds can be detected and quantified directly from solution; there is no need to volatilize the sample. The technique offers excellent low sensitivity (femtomole detection limits). Because compounds in the mixture are separated by mass spectrometry instead of by chromatography, the entire process, from ionization and sample injection to data acquisition by computer, takes only seconds.

The computer data can be analyzed in several ways. One can use a *parent ion* mode to obtain an array of all parent ions that fragment to produce a particular daughter ion, or a *neutral loss* mode to obtain an array of all parent ions that lose a common neutral fragment. Further, these *scan functions* can be changed many times during analysis, so that one can detect and measure butyl esters of acylcarnitines (by the signature ion at m/z 85) and the butyl esters of α-amino acids (by loss of a neutral 102 fragment) in the same sample.

MS/MS permits very rapid, sensitive, and, with appropriate internal standards, accurate measurement of many different types of metabolites with minimal sample preparation and without prior chromatographic separation. Because many amino acidemias, organic acidemias, and disorders of fatty acid oxidation can be detected in one to two minutes, the system has adequate throughput to handle the large number of samples that are processed in newborn screening programs (*159*). Some conditions that can be diagnosed by MS/MS are listed in Table IX-1, together with the compound(s) on which diagnosis is based (*160–165*).

It is important to note that MS/MS cannot replace current programs to screen for biotinidase deficiency, hypothyroidism, hemoglobinopathies, virilizing adrenal hyperplasia, and galactosemia; these conditions cannot be identified by MS/MS at this time and must be detected by other means.

Table IX-1.

Some Disorders Detectable by Tandem Mass Spectrometry (*158,160–165*)

Disorder	Diagnostic metabolite
Amino acidemias	
Phenylketonuria	Phenylalanine and tyrosine
Maple syrup urine disease	Leucine and isoleucine
Homocystinuria (CBS deficiency)	Methionine
Citrullinemia	Citrulline
Hepatorenal tyrosinemia	Methionine and tyrosine
Organic acidemias	
Propionic acidemia	C3 acylcarnitine
Methylmalonic acidemia(s)	C3 acylcarnitine
Isovaleric acidemia	Isovalerylcarnitine
Isolated 3-methylcrotonylglycinemia	3-Hydroxyisovalerylcarnitine
Glutaric acidemia (type I)	Glutarylcarnitine
Hydroxymethylglutaric acidemia	Hydroxymethylglutarylcarnitine
Fatty acid oxidation disorders	
SCAD deficiency	C4,6 acylcarnitines
MCAD deficiency	C8,10:1 acylcarnitines
VLCAD deficiency	C14,14:1,16,18 acylcarnitines
LCHAD and trifunctional protein deficiency	C14,14:1,16,18 acyl- and 3-hydroxy acylcarnitines
Glutaric acidemia type II	Glutarylcarnitine
CPT-II deficiency	C14,14:1,16,16:1 acylcarnitines

.

SECTION X.

Recommendations for the Measurement of Urine Organic Acids

The measurement of urine organic acids is an important component of the investigation of inherited metabolic disease. If utilized appropriately, this one assay is capable of identifying abnormal metabolic profiles that occur in approximately 150 distinct genetic disorders. A significant number of metabolic diseases can only be identified using this procedure. Early diagnosis before repeated episodes of metabolic decompensation occur is likely to result in better patient outcome for a number of disorders. For other currently untreatable conditions, early diagnosis enables genetic counseling to be provided before multiple affected siblings are delivered.

GUIDELINE 38: Urine organic acid analysis.

Urine organic acid analysis using the procedures identified below should be made readily available to all patients (children and adults) in whom a metabolic disease is suspected.

Preanalytical Concerns

Time of sample collection. Many disorders of organic acid metabolism present with abnormal metabolite profiles at all stages of clinical severity. These disorders should be readily identifiable in affected patients irrespective of sample collection time. However, some disorders of energy metabolism only present with abnormal organic acid profiles during periods of metabolic decompensation. Samples collected after the acute illness may not demonstrate significant abnormalities for these patients and the diagnosis may be missed. Frequently, samples of urine are collected in the emergency room for infection and toxicology investigations from patients with metabolic decompensation.

Concurrent therapies. Certain therapeutic modalities can produce urine organic acid profiles that may mask underlying metabolic disease. Examples of therapeutic interference include seizure treatment with valproic acid and caloric supplementation with medium-chain triglycerides. If an acceptable infectious or toxicological etiology for the acute presentation is identified, metabolic studies including urine organic acid analysis may not be necessary.

GUIDELINE 39: Urine collection.

Therefore, we recommend that whenever possible urine for organic acid analysis should be collected from patients at the same time.

Sample storage. Urine organic acids are stable for long periods of time (several years) if stored at -70 ˚C and for several months at -20 ˚C.

Guideline 40: Sample storage.

Samples should be stored at -20 ˚C prior to analysis unless analysis is immediate, in which case freezing is not necessary.

Analytical concerns. The only acceptable method of analysis for urine organic acids is by capillary gas chromatography-mass spectrometry.

Sample preparation. A volume of thawed, thoroughly mixed urine equivalent to a constant amount of creatinine is aliquoted for extraction. This is typically the equivalent volume containing around 1–2 µmol (0.1–0.2 mg) of creatinine. For most samples this yields between 0.5 and 3.0 mL of urine to be extracted. For extremes of concentration, we recommend that the minimum volume to be extracted is 0.5 mL and the maximum is 3.0 mL.

To this volume of urine, a fixed volume of internal standard is added. It is also acceptable to aliquot a fixed amount of urine and add to it a variable amount of internal standard to achieve the same ratio of the two components. The internal standard chosen should not be a metabolite that might be detected in normal or pathological urine, nor should it co-chromatograph with significant metabolites. Typical internal standards include heptadecanoic acid, 2-phenylbutyric acid, and dimethylmalonic acid. The final concentration of internal standard should be chosen to generate a peak on the total ion chromatogram that is similar in height to the highest detected organic acids.

Oximation. The addition of an oximating regent such as ethoxylamine hydrochloride serves to preserve ketoacids that are present in urine. Important ketoacids include the 2-ketoisocaproic, 2-keto-3-methylvaleric, and 2-ketoisovaleric acids present in maple syrup urine disease. In the absence of oximation, a significant proportion of ketoacids is converted to the corresponding 2-hydroxyacid. The substance 2-hydroxyisovaleric acid is an important indicator of maple syrup urine disease, which is readily identified in non-oximated urine samples.

Method of sample extraction. Urine plus internal standard should be acidified to pH 1–2 and extracted into an equal volume of an organic solvent. Ethyl acetate extraction is most commonly employed. The sample may be extracted up to three times for greatest efficiency. The addition of saturating amounts of sodium chloride prior to the extraction process may reduce the extraction efficiency of urea, which can interfere with the identification of other organic acids. Solid phase extraction using silicic acid minicolumns has also been employed successfully for sample extraction. We recommend that information regarding all concurrent therapies be provided with the patient order for urine organic acid analysis.

Method of sample derivatization. Most databases for organic acid spectra are based upon spectra generated from trimethylsilyl (TMS)-derivatives.

Gas Chromatography-Mass Spectrometry

GUIDELINE 41: TMS derivatization.

TMS derivatives of extracted urinary organic acids should be prepared for GC-MS analysis.

Instrument tuning. It is critical for mass assignment to ensure that the analyzer is tuned regularly. Most bench-top GC-MS systems have an auto-tune capability.

Guideline 42: GC-MS instrument tuning.

- An instrument auto-tune should be performed daily.
- Analysis should only proceed if the tune falls within the specifications provided by the instrument manufacturer.

Choice of column. A variety of capillary GC columns are used to separate organic acids with equivalent efficiency of separation. Columns are typically 25–30 meters in length, 0.2–0.5 mm in internal diameter, and coated with a 0.1–1.0 μm layer of an OV1, OV5, or OV17 comparable liquid coating. Each manufacturer has a proprietary brand. Overloading the column can cause difficulty in peak identification.

GUIDELINE 43: Sample injection.

Sample injection onto the column should be in the split mode with a 1–2 μL injection and a split ratio of at least 1:15 to prevent column overload.

Running conditions. A temperature ramp is important to elute organic acids with low volatility. These are typical and recommended GC temperatures: injection port 240–250 ˚C; initial oven temperature 70–100 ˚C; temperature ramp 3–8 ˚C per minute; final oven temperature 270–295 ˚C.

GUIDELINE 44: Column temperature.

- The temperature of the mass spectrometer interface should be equal to or greater than the highest column temperature.
- The initial oven temperature, rate of temperature ramp, and highest temperature will determine the total run time, which is typically 30–60 min.

Data acquisition. Data acquisition in the mass spectrometer should not begin until the solvent front has returned to the baseline. Data should then be acquired in scan mode with a full-scale scan every 0.5 seconds.

GUIDELINE 45: Data acquisition.

Depending upon the mass range of the mass spectrometer, the range of ions scanned should be from m/z 50 to m/z 500–650. This data should be presented as a total ion chromatogram.

Peak identification. Peaks should be identified both by retention time and by spectral match in an appropriate library of TMS-derivative spectra. Spectral match should be greater than 80% in the presence of a known co-chromatographing peak to provide positive identification. Several commercial libraries are available for purchase but we recommend that centers measuring urinary organic acids also build their own in-house library based upon experience and availability of samples from patients with organic acidurias.

Calibration. The analytical system should be calibrated using a solution of multiple organic acids of known concentration that elute at various points during the chromatographic run.

GUIDELINE 46: Calibration.

- We recommend that 10–15 analytes be used in this calibrator mix and that they consist of significant compounds of diagnostic interest.
- Standard curves encompassing the reportable range for an analyte should be generated at frequent intervals.

Data interpretation. Regarding quantitative versus qualitative data analysis: some laboratories provide extensive quantitative reports while others generate a qualitative interpretation. There is no consensus as to which format is most favorable.

For quantitative reporting, most analytes are quantified as a unique ion ratio for that compound to an ion specific to the internal standard.

GUIDELINE 47: For concentrations of organic acids less than 100 mmol/mol creatinine:

Quantitation should be by isotope ratio mass spectrometry using stable isotope-labeled internal standards.

Data collection for this purpose should be in the selected ion mode, using at least two ions for both internal standard and native compound. Experience in interpreting both quantitative and qualitative reports is essential. The rarity of some organic acidurias means that very few laboratories have a great depth of experience.

GUIDELINE 48: Proficiency challenges.

Laboratories measuring urine organic acids should participate in proficiency activities, e.g., CAP, and in addition, should also exchange abnormal samples to extend their experience.

Identification of minor pathological components. We recognize that there are some urine organic acid components that have critical diagnostic value but are only present in small amounts, often hidden in the background noise. These components may be identified in a total ion chromatogram if selected ions are investigated. Compounds that should be sought in all organic acid chromatograms include the following:

1. n-Hexanoylglycine, an important marker of medium-chain acyl CoA dehydrogenase deficiency.
2. Ethylmalonate, a marker for multiple disorders, frequently co-chromatographs with phosphate, which is quantitatively a more significant compound.
3. Orotic acid, a marker for a number of urea cycle disorders, which frequently co-chromatographs with aconitate.
4. 4-Hydroxybutyrate (gamma hydroxybutyrate), a marker for succinic semialdehyde dehydrogenase deficiency.
5. 3-Hydroxyglutarate, a marker for glutaric acidemia type 1.

References

1. Health Canada. Perinatal health indicators for Canada: a resource manual. Ottawa, Canada: Minister of Public Works and Government Services, 2000.
2. Enkin M, Kerise M, Neilson J, et al. A guide to effective care in pregnancy and childbirth, 3rd ed. Oxford: Oxford University Press, 2000.
3. Lockitch G. Clinical biochemistry of pregnancy. Crit Rev Clin Lab Sci 1997;34:67.
4. Lockitch G, ed. Handbook of diagnostic biochemistry and hematology in normal pregnancy. Boca Raton: CRC Press, 1993.
5. Ramsay MM. Normal values in pregnancy. London: WB Saunders, 1996.
6. Gronowski A, ed. Handbook of clinical laboratory testing during pregnancy. New York: Humana Press, 2004.
7. Munoz FM, Englund JA. Vaccines in pregnancy. Inf Dis Clin N Am 2001;15:253–271.
8. Stevenson AM. Immunizations for women and infants. J Ob Gyn Neonat Nurs 1999;28:534-544.
9. Lutwick LI. Unconventional vaccine targets. Immunization for pregnancy, peptic ulcer, gastric cancer, cocaine abuse, and atherosclerosis. Inf Dis Clin N Amer 1999;13:245–264.
10. Englund J, Glezen WP, Piedra PA. Maternal immunization against viral disease. Vaccine 1998;16:1456–1463.
11. Glezen WP, Alpers N. Maternal immunization. Clin Infect Dis 1999;28:219–224.
12. Global programme for vaccines and immunization. Programme report 1995, WHO/GPV/96.0l. Geneva: World Health Organization, 1996.
13. Neuzil KM, Reed GW, Mitchel EF Jr, et al. Influenza-associated morbidity and mortality in young and middle-aged women. JAMA 1999;281:901–907.
14. Centers for Disease Control Advisory Committee on Immunization Practices. Prevention and control of influenza. MMWR Morb Mortal Wkly Rep 2000;48:1–28.
15. Centers for Disease Control and Prevention. Poliomyelitis prevention in the United States: updated recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 2000;49:1–22.
16. American Academy of Pediatrics. Poliovirus infections. In: Pickering LK, ed. 2000 red book report of the Committee on Infectious Diseases, 25th ed. Elk Grove Village, IL: American Academy of Pediatrics, 2000:465–470.
17. Tsai TF, Paul R, Lynberg MC, et al. Congenital yellow fever virus infection after immunization in pregnancy. J Infect Dis 1993;168:1520–1523.
18. O'Dempsey TJ, McArdle T, Ceesay SJ, et al. Meningococcal antibody titers in infants of women immunized with meningococcal polysaccharide vaccine during pregnancy. Arch Dis Child Fetal Neonatal Ed 1996;74:F43–6.
19. Sahid NO, Steinhoff MC, Hoque SS, et al. Serum, breast milk, and infant antibody after maternal immunisation with pneumococcal vaccine. Lancet 1995;346:1252–1257.
20. Ray JG. Lues-lues: maternal and fetal considerations of syphilis. Obstet Gynecol Surv 1995;50:845–850.
21. Watson JC, Hadler SC, Dykewicz CA, Reef S, Phillips L. Measles, mumps, and rubella vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 1998;47:1–57.
22. Boxall E. Screening of pregnant women for hepatitis B. Vaccine 1998;16:530–533.
23. Burns DN, Minkoff H. Hepatitis C: screening in pregnancy. Obstet Gynecol 1999;94:1044–1048.
24. Human immunodeficiency virus screening. Joint statement of the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists. Pediatrics 1999;104–128.
25. Gilbert RE, Gupta AR, Ades AE, Logan S, Sculpher M, van der Meulen JHP. Screening for Down syndrome: effects, safety, and cost effectiveness of first and second trimester strategies. BMJ 2001;323:423–425.
26. Hyett J, Perdu M, Sharland G, Snijders R, Nicolaides KH. Using fetal nuchal translucency to screen for major congenital cardiac defects at 10–14 weeks of gestation: population-based cohort study. BMJ 1999;318:81–85.

27. Pandya PP, Snidjers RJM, Johnson SP, Brizot MDL, Nicolaides KH. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. Br J Obstet Gynaecol 1995;102:957–962.

28. Souka AP, Snidjers RJM, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10–14 weeks of gestation. Ultrasound Obstet Gynecol 1998;11:391–400.

29. Krantz DA, Larsen JW, Buchanan PD, Macri JN. First-trimester Down syndrome screening: free beta human chorionic gonadotropin and pregnancy-associated plasma protein A. Am J Obstet Gynecol 1996;174:612–616.

30. Spencer K, Tul N, Nicolaides KH. Maternal serum free β-hCG and PAPP-A in fetal sex chromosome defects in the first trimester. Prenat Diagn 2000;20:411–416.

31. Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with Trisomy 21 at 11–14 weeks of gestation: an observational study. Lancet 2001;358:1665–1667.

32. Tul N, Spencer, K Noble P. Screening for Trisomy 18 by fetal nuchal translucency and maternal serum free beta hCG and PAPP-A at 10–14 weeks of gestation. Prenat Diagn 1999;19:1035–1042.

33. Wren J, Craven B. A cost-effectiveness study of changing medical practice in early pregnancy. Clin Perform Qual Healthcare 1999;7:172–177.

34. Luciano AA, Roy G, Solima E. Ectopic pregnancy from surgical emergency to medical management. Ann NY Acad Sci 2001;943:235–254.

35. Lipscomb GH, Stovall TG, Ling FW. Nonsurgical treatment of ectopic pregnancy. N Engl J Med 2000;343:1325–1329.

36. Cuckle H, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy with Down syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94:387–402.

37. Palomaki GE, et al. Risk-based screening for Trisomy 18 using alpha-fetoprotein, unconjugated oestriol, and human chorionic gonadotropin. Prenatal Diagnosis 1995;15:713–723.

38. Wald NJ, Kennard A, et al. Antenatal screening for Down's syndrome [review]. J Med Screening 1997:4(4):181–246. (Section 3 on second–trimester serum markers contains a bibliography of 149 items.)

39. Wald NJ, Brock DJH, Bonnar J. Prenatal diagnosis of spina bifida and anencephaly by maternal serum alpha-fetoprotein measurement. Lancet 1974;1:765–767.

40. Haddow JE, Palomaki GE, et al. Prenatal screening for Down syndrome with use of maternal serum markers. N Engl J Med 1992; 327:588–593.

41. Wald NJ, Cuckle HS. The quality control of alpha-fetoprotein reagents and assay for the antenatal screening and diagnosis of open neural-tube defects. Report of a workshop sponsored by the National Institute of Child Health and Human Development. Clin Chem Acta 1980;105:9–24.

42. CEMACH. Why mothers die, 1997–1999: the confidential enquiries into maternal deaths in the UK. London: RCOG Press, 2001.

43. Rey E, LeLorier J, Burgess E, Lange IR, Leduc L. Report of the Canadian Hypertension Society Consensus Conference: 3. Pharmacologic treatment of hypertensive disorders in pregnancy. CMAJ 1997;157(9):1245–1254.

44. Report of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183(1):S1–S22.

45. Brown MA, Hague WM, Higgins J, Lowe S, McCowan L, Oats J, et al. The detection, investigation, and management of hypertension in pregnancy: executive summary. Aust N Z J Obstet Gynaecol 2000;40(2):133–138.

46. Douglas KA, Redman CWG. Eclampsia in the United Kingdom—Reply. Br Med J 1995;310(6987):1138.

47. Caritis S, Sibai B, Hauth J, Lindheimer MD, Klebanoff M, Thom E,, et al. Low-dose aspirin to prevent preeclampsia in women at high risk. New Engl J Med 1998;338(11):701–705.

48. Mackay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. Obstet Gynecol 2001;97(4):533–538.

49. Stevenson DK, Wright LL, Lemons JA, Oh W, Korones SB, Papile LA, et al. Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, January 1993 through December 1994. Am J Obstet Gynecol 1998;179:1632–1639.

50. von Dadelszen , Magee LA, Lee SK, Stewart SD, Simone C, Koren G, et al. Activated protein C in normal human pregnancy and pregnancies complicated by severe preeclampsia: a therapeutic opportunity? Crit Care Med 2002;30(8):1883–1892.

51. Saudan PJ, Brown MA, Farrell T, Shaw L. Improved methods of assessing proteinuria in hypertensive pregnancy. Brit J Obstet Gynaec 1997;104(10):1159–1164.

52 Saudan PJ, Brown MA, Farrell T. Spot urine protein-to-creatinine ratio for assessing proteinuria in hypertensive pregnancies. Kidney Int 1997;51(4):1306.

53. Waugh J, Bell SC, Kilby M, Lambert P, Shennan A, Halligan A. Effect of concentration and biochemical assay on the accuracy of urine dipsticks in hypertensive pregnancies. Hypertens Pregnancy 2001;20(2):205–217.

54. Caetano M, Ornstein M, von Dadelszen P, Hannah ME, Logan AG, Gruslin A, Willan A, Magee LA. A survey of Canadian practitioners regarding the diagnosis and evaluation of the hypertensive disorders of pregnancy. Hypertens Preg (MS#2002–70, in press).

55. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long-term mortality of mothers and fathers after preeclampsia: population-based cohort study. Br Med J 2001;323(7323):1213–1216.

56. Greer IA. Thrombosis in pregnancy: maternal and fetal issues. Lancet 1999;353(9160):1258–1265.

57. Macklon NS, Greer IA. Venous thromboembolic disease in obstetrics and gynaecology: the Scottish experience. Scott Med J 1996;41(3):83–86.

58. Report of the RCOG working party on prophylaxis against thromboembolism in gynaecology and obstetrics. London: Royal College of Obstetricians and Gynaecologists, 1995.

59. Laffan M, Tuddenham E. Science, medicine, and the future—assessing thrombotic risk. Br Med J 1998;317(7157):520–523.

60. Brill–Edwards P, Ginsberg JS, Gent M, Hirsh J, Burrows R, Kearon C, et al. Safety of withholding heparin in pregnant women with a history of venous thromboembolism. New Engl J Med 2000;343(20):1439–1444.

61. Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). Br Med J 1997;314(7076):253–257.

62. Martin JA, Hamilton BE, Ventura SJ, Menacker F, Park MM. Births: final data for 2000. National vital statistics reports 5. Hyattsville, MD: National Center for Health Statistics, 2002;50(5).

63. Miniño AM, Arias E, Kochanek KD, Murphy SL, Smith BL. Deaths: final data for 2000. National vital statistics reports. Hyattsville, MD: National Center for Health Statistics, 2002;50(15).

64. Goldenberg RL, Mercer BM, Iams JD, Moawad AH, Meis PJ, Das A, et al. The preterm prediction study: patterns of cervicovaginal fetal fibronectin as predictors of spontaneous preterm delivery. National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. Am J Obstet Gynecol 1997;177(1):8–12.

65. Joffe GM, Jacques D, Bemis-Heys R, Burton R, Skram B, Shelburne P. Impact of the fetal fibronectin assay on admissions for preterm labor. Am J Obstet Gynecol 1999;180(3 Pt 1):581–586.

66. Iams JD, Casal D, McGregor JA, Goodwin TM, Kreaden US, Lowensohn R, et al. Fetal fibronectin improves the accuracy of diagnosis of preterm labor. Am J Obstet Gynecol 1995;173(1):141–145.

67. Peaceman AM, Andrews WW, Thorp JM, Cliver SP, Lukes A, Iams JD, et al. Fetal fibronectin as a predictor of preterm birth in patients with symptoms: a multicenter trial. Am J Obstet Gynecol 1997;177(1):13–18.

68. Luzzi V, Hankins K, Gronowski AM. Accuracy of the rapid fetal fibronectin TLi system in predicting preterm delivery. Clin Chem 2003;49(3):501–502.

69. Sullivan A, Hueppchen NA, Satin AJ. Cost effectiveness of bedside fetal fibronectin testing varies according to treatment algorithm. J Matern Fetal Med 2001;10(6):380–384.

70. Kiss H, Ahner R, Hohlagschwandtner M, Leitich H, Husslein P. Fetal fibronectin as a predictor of term labor: a literature review. Acta Obstet Gynecol Scand 2000 Jan;79(1):3–7.

71. Bishop EH. Pelvic scoring for elective induction. Obstet Gynecol 1964;24:266–268.

72. Williams MC, Krammer J, O'Brien WF. The value of the cervical score in predicting successful outcome of labor induction. Obstet Gynecol 1997;90(5):784–789.

73. Hedriana HL, Munro CJ, Eby-Wilkens EM, Lasley BL. Changes in rates of salivary estriol increases before parturition at term. Am J Obstet Gynecol 2001;184(2):123–130.

74. Goffinet F, Maillard F, Fulla Y, Cabrol D. Biochemical markers (without markers of infection) of the risk of preterm delivery. Implications for clinical practice. Eur J Obstet Gynecol Reprod Biol 2001;94(1):59–68.

75. Goldenberg RL, Iams JD, Mercer BM, Meis PJ, Moawad A, Das A, et al. The preterm prediction study: toward a multiple-marker test for spontaneous preterm birth. Am J Obstet Gynecol 2001;185(3):643–651.

76. Oulton M, Martin TR, Faulkner GT, Stinson D, Johnson JP. Developmental study of a lamellar body fraction isolated from human amniotic fluid. Pediatr Res 1980;14:722–728.

77. Hawgood S, Clements JA. Pulmonary surfactant and its apoproteins. J Clin Invest 1990;86:1–6.

78. Persson A, Chang D, Crouch E. Surfactant protein D is a divalent cation-dependent carbohydrate-binding protein. J Biol Chem 1990;26510:5755–5760.

79. Moore TR. A comparison of amniotic fluid fetal pulmonary phospholipids in normal and diabetic pregnancy. Am J Obstet Gynecol 2002;186:641–650.

80. American College of Obstetricians and Gynecologists, Committee on Educational Bulletins. Assessment of fetal lung maturity. ACOG Educational Bulletin No. 230. Washington, DC: American College of Obstetricians and Gynecologists, 1996.

81. College of American Pathologists. CAP surveys, lung maturity survey, set LM–B. Northfield, IL: College of American Pathologists, 2002.

82. College of American Pathologists. Supplemental questions on lamellar body counts. Surveys 000;LM–C:4–5.

83. Ashwood ER, Tait JF, Foerder CA, Franklin RW, Benedetti TJ. Improved fluorescence polarization assay for use in evaluating fetal lung maturity. III. Retrospective clinical evaluation and comparison with the lecithin/sphingomyelin ratio. Clin Chem 1986;32:260–264.

84. Tsai MY, Shultz EK, Williams PP, Bendel R, Butler J, Farb H, et al. Assay of disaturated phosphatidylcholine in amniotic fluid as a test of fetal lung maturity: experience with 2000 analyses. Clin Chem 1987;33(9):1648–1651.

85. Ashwood ER, Palmer SE, Taylor JS, Pingree SS. Lamellar body counts for rapid fetal lung maturity testing. Obstet Gynecol 1993;81:619–624.

86. Bender TM, Stone LR, Amenta JS. Diagnostic power of lecithin/sphingomyelin ratio and fluorescence polarization assays for respiratory distress syndrome compared by relative operating characteristic curves. Clin Chem 1994;40(4):541–545.

87. Wijnberger LD, Huisjes AJ, Voorbij HA, Franx A, Bruinse HW, Mol BW. The accuracy of lamellar body count and lecithin/sphingomyelin ratio in the prediction of neonatal respiratory distress syndrome: a meta-analysis. BJOG 2001;108(6):583–588.

88. Tait JF, Foerder CA, Ashwood ER, Benedetti TJ. Prospective clinical evaluation of an improved fluorescence polarization assay for predicting fetal lung maturity. Clin Chem 1987;33:554–558.

89. Chen C, Roby PV, Weiss NS, Wilson JA, Benedetti TJ, Tait JF. Clinical evaluation of the NBD-PC fluorescence polarization assay for prediction of fetal lung maturity. Obstet Gynecol 1992;80:688–692.

90. Ruch AT, Lenke RR, Ashwood ER. Assessment of fetal lung maturity by fluorescence polarization in high-risk pregnancies. J Reprod Med 1993;38:133–136.

91. Fantz CR, Powell C, Karon B, Parvin CA, Hankins K, Dayal M, et al. Assessment of the diagnostic accuracy of the TDx-FLM II to predict fetal lung maturity. Clin Chem 2002;48:761–765.

92. Kesselman EJ, Figueroa R, Garry D, Maulik D. The usefulness of the TDx/TDxFLx fetal lung maturity II assay in the initial evaluation of fetal lung maturity. Am J Obstet Gynecol 2003;188:1220–1222.

93. Neerhof MG, Dohnal JC, Ashwood ER, Lee IS, Anceschi MM. Lamellar body counts: a consensus on protocol. Obstet Gynecol 2001;97(2):318–320.

94. Dubin SB: Characterization of amniotic fluid lamellar bodies by resistive-pulse counting: Relationship to measures of fetal lung maturity. Clin Chem 1989;35:612–616.

95. Greenspoon JS, Rosen DJ, Roll K, Dubin SB. Evaluation of lamellar body number density as the initial assessment in a fetal lung maturity test cascade. J Reprod Med 1995;40:260–266.

96. Bowie LJ, Shammo J, Dohnal JC, Farrell E, Vye MV. Lamellar body number density and the prediction of respiratory distress. Am J Clin Pathol 1991;95:781–786.

97. Dalence CR, Bowie LJ, Dohnal JC, Farrell EE, Neerhof MG. Amniotic fluid lamellar body count: a rapid and reliable fetal lung maturity test. Obstet Gynecol 1995;86:235–239.

98. Fakhoury G, Daikoku NH, Benser J, Dubin NH. Lamellar body concentrations and the prediction of fetal pulmonary maturity. Am J Obstet Gynecol 1994;170:72–76.

99. Lee IS, Cho YK, Kim A, Min WK, Kim KS, Mok JE. Lamellar body count in amniotic fluid as a rapid screening test for fetal lung maturity. J Perinatol 1996;16:176–180.

100. Pearlman ES, Baiocchi JM, Lease JA, Gilbert J, Cooper JH. Utility of a rapid lamellar body count in the assessment of fetal maturity. Am J Clin Pathol 1991;95:778–780.

101. Dilena BA, Ku F, Doyle I, Whiting MJ. Six alternative methods to the lecithin/sphingomyelin ratio in amniotic fluid for assessing fetal lung maturity. Ann Clin Biochem 1997;34:106–108.

102. Neerhof MG, Haney EI, Silver RK, Ashwood ER, Lee IS, Piazze JJ. Lamellar body counts compared with traditional phospholipid analysis as an assay for evaluating fetal lung maturity. Obstet Gynecol 2001;97:305–309.

103. Beinlich A, Fischass C, Kaufmann M, Schlosser R, Dericks-Tan JS. Lamellar body counts in amniotic fluid for prediction of fetal lung maturity. Arch Gynecol Obstet 1999;262:173–180.

104. Sher G, Statland BE, Freer DE, Kraybill EN. Assessing fetal lung maturation by the foam stability index test. Obstet Gynecol 1978;52:673–677.

105. Sher G, Statland BE, Freer DE. Clinical evaluation of the quantitative foam stability index test. Obstet Gynecol 1980;55:617–620.

106. Sher G, Statland BE, Knutzen VK. Diagnostic reliability of the lecithin/sphingomyelin ratio assay and the quantitative foam stability index test: results of a comparative study. J Reprod Med 1982;27:51–55.

107. Sher G, Statland BE. Assessment of fetal pulmonary maturity by the Lumadex foam stability index test. Obstet Gynecol 1983;61:444–449.

108. Lockitch G, Wittmann BK, Snow BE, Campbell DJ. Prediction of fetal lung maturity by use of the Lumadex-FSI test. Clin Chem 1986;32:361–363.

109. Lipshitz J, Whybrew WD, Anderson GD. Comparison of the Lumadex-foam stability index test, lecithin: sphingomyelin ratio, and simple shake test for fetal lung maturity. Obstet Gynecol 1984;63:349–354.

110. Halvorsen PR, Gross TL. Laboratory and clinical evaluation of a rapid slide agglutination test for phosphatidylglycerol. Am J Obstet Gynecol 1985;151:1061–1066.

111. Weinbaum PJ, Richardson D, Schwartz JS, Gabbe SG. Amniostat FLM: a new technique for detection of phosphatidylglycerol in amniotic fluid. Am J Perinatol 1985;2:88–92.

112. Lockitch G, Wittmann BK, Mura SM, Hawkley LC. Evaluation of the amniostat-FLM assay for assessment of fetal lung maturity. Clin Chem 1984;30:1233–1237.

113. Garite TJ, Yabusaki KK, Moberg LJ, Symons JL, White T, Itano M, et al. A new rapid slide agglutination test for amniotic fluid phosphatidylglycerol: laboratory and clinical correlation. Am J Obstet Gynecol 1983;147:681–686.

114. Towers CV, Garite TJ. Uselessness of the phosphatidylglycerol assay for prediction of lung maturity (reply). Am J Obstet Gynecol 1989;161:1419.

115. Dubin SB. Assessment of fetal lung maturity. Practice parameter. Am J Clin Pathol 1998;110:723–732.

116. Szallasi A, Gronowski AM, Eby CS. Lamellar body count in amniotic fluid: a comparative study of four different hematology analyzers. Clin Chem 2003;49:994–997.

117. Farquharson J, Jamieson EC, Berry E, Buchanan R, Logan RW. Assessment of the amniostat-FLM immunoagglutination test for phosphatidylglycerol in amniotic fluid. Clin Chim Acta 1986;156:271–277.

118. Benoit J, Merrill S, Rundell C, Meeker CI. Amniostat-FLM: an initial clinical trial with both vaginal pool and amniocentesis samples. Am J Obstet Gynecol 1986;154:65–68.

119. Grenache DG, Parvin CA, Gronowski AM. Preanalytical factors that influence the Abbott TDx Fetal Lung Maturity II assay. Clin Chem 2003;49:935–939.

120. Lenke R, Ashwood E. Lung Maturity Testing. In: Quilligan EJ, Zuspan FP, eds. Current therapy in obstetrics and gynecology, 5th ed. Philadelphia: WB Saunders, 2000:419.

121. Longo SA, Towers CV, Strauss A, Asrat T, Freeman RK. Meconium has no lecithin or sphingomyelin but affects the lecithin/sphingomyelin ratio. Am J Obstet Gynecol 1998;179:1640–1642.

122. Weitzner JS, Strassner HT, Rawlins RG, Mack SR, Anderson RA Jr. Objective assessment of meconium content of amniotic fluid. Obstet Gynecol 1990;76(6):1143–1144.

123. Tait JF, Franklin RW, Simpson JB, Ashwood ER. Improved fluorescence polarization assay for use in evaluating fetal lung maturity: I. Development of the assay procedure. Clin Chem 1986;32:248–254.

124. Robert MF, Neff RK, Hubbell JP, Taeusch HW, Avery ME. Association between maternal diabetes and the respiratory distress syndrome in the newborn. N Engl J Med 1976;294:357–360.

125. Mimouni F, Miodovnik M, Whitsett JA, Holroyde JC, Siddiqi TA, Tsang RC. Respiratory distress syndrome in infants of diabetic mothers in the 1980s: no direct adverse effect of maternal diabetes with modern management. Obstet Gynecol 1987;69:191–195.

126. Cruz AC, Buhi WC, Birk SA, Spellacy WN. Respiratory distress syndrome with mature lecithin/sphingomyelin ratios: diabetes mellitus and low Apgar scores. Am J Obstet Gynecol 1978;126:78–82.

127. Gabbe SG, Lowensohn RI, Mestman JH, Freeman RK, Goebelsmann U. Lecithin/sphingomyelin ratio in pregnancies complicated by diabetes mellitus. Am J Obstet Gynecol 1977;128:757–760.

128. Tabsh KM, Brinkman CR III, Bashore RA. Lecithin:sphingomyelin ratio in pregnancies complicated by insulin-dependent diabetes mellitus. Obstet Gynecol 1982;59:353–358.

129. Piper JM, Langer O. Does maternal diabetes delay fetal pulmonary maturity? Am J Obstet Gynecol 1993;168:783–786.

130. Berkowitz K, Reyes C, Saadat P, Kjos SL. Fetal lung maturation. Comparison of biochemical indices in gestational diabetic and nondiabetic pregnancies. J Reprod Med 1997;42:793–800.

131. Del Valle GO, Adair CD, Ramos EE, Gaudier FL, Sanchez-Ramos L, Morales R. Interpretation of the TDx-FLM fluorescence polarization assay in pregnancies complicated by diabetes mellitus. Am J Perinatol 1997;14:241–244.

132. Livingston EG, Herbert WN, Hage ML, Chapman JF, Stubbs TM. Use of the TDx-FLM assay in evaluating fetal lung maturity in an insulin-dependent diabetic population. The Diabetes and Fetal Maturity Study Group. Obstet Gynecol 1995;86:826–829.

133. Tanasijevic MJ, Winkelman JW, Wybenga DR, Richardson DK, Greene MF. Prediction of fetal lung maturity in infants of diabetic mothers using the FLM S/A and disaturated phospatidylcholine tests. Am J Clin Pathol 1996;105:17–22.

134. Tsai MY, Shultz EK, Nelson JA. Amniotic fluid phosphatidylglycerol in diabetic and control pregnant patients at different gestational lengths. Am J Obstet Gynecol 1984;149:388–392.

135. Cunningham MD, McKean HE, Gillispie DH, Greene JW. Improved prediction of fetal lung maturity in diabetic pregnancies: a comparison of chromatographic methods. Am J Obstet Gynecol 1982;142:197–204.

136. Whitworth NS, Magann EF, Morrison JC. Evaluation of fetal lung maturity in diamniotic twins. Am J Obstet Gynecol 1999;180:1438–1441.

137. Larrson L. Technology and the future of neonatal testing. EduTrak presentation #3405. AACC Annual Meeting, San Francisco, CA, July 26, 2000.

138. Goldsmith BM. Clinical chemistry of the newborn and infant. Lab Med 1997;28:659–663.

139. Goldsmith BM. STAT testing in the neonate. Blood Gas News 2002;11(1):4–13.

140. Young DS. Preanalytical issues in neonatology. Blood Gas News 2002;11(1):14–18.

141. Pishva N, Madani A, Homayoon K. Prophylactic intravenous immunoglobulin in neonatal immune hemolytic jaundice. Irn J Med Sci 2000;25(3&4):129–133.

142. Hawdon JM. Glucose and lactate in neonatology (clinical focus). Blood Gas News 2002;11(6):37–40.

143. Tang Z, Kost GJ. Reducing errors in glucose meter measurements. Advance 2000;9(8):10–11.

144. Centers for Disease Control and Prevention. Prevention of perinatal Group B streptococcal disease. MMWR 2002;51(No. RR–11):1–22.

145. Schrag SJ, Zywicki S, Farley MM, Reingold AL, Harrision LH, Lefkowitz LB, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med 2000;342:15–20.

146. Chiesa C, Pellegrinin G, Panero A, Osborn J, Signore F, Assumma M, et al. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period. Influence of illness severity, risk status, antenatal, and perinatal complications and infection. Clin Chem 2003;49(1);60–68.

147. Garg SK. The utility of serial C-reactive proteins in the newborn as an indicator for neonatal depsis. Diabetes Care 1999;22:1708–1714.

148. Weiss IK, Fink S, Harrison R, Feldman JD, Brill JE. Clinical assessment: continuous arterial blood gas monitoring in the pediatric intensive care unit. Pediatrics 1999;103:440–445.

149. Widness JA, Kulhavy JC, Johnson KJ, Cress GA, Kromer IJ, Acarregui MJ, et al. Clinical performance of an in-line point-of-care monitor in neonates. Pediatrics 2000;106:497–504.

150. Bhutani VK, Gourley GR, Adler S, Kreamer B, Dalin C, Johnson LH. Noninvasive measurement of total serum bilirubin in a multiracial predischarge newborn population to assess the risk of severe hyperbilirubinemia. Pediatrics 2000;106:2–17.

151. Green N. Professional consumer and public health perspectives in newborn screening programs. Presentation by March of Dimes. AACC symposium on advances in newborn screening, Lexington, KY, May 2002.

152. Lloyd-Puryear MA. MCHB current and future priorities around newborn screening. Presentation by Health Resources and Services Administration Maternal and Child Health Bureau. AACC symposium on advances in newborn screening, Lexington, KY, May 2002.

153. Chaing SH. Newborn screening program in North Carolina. AACC symposium on advances in newborn screening, Lexington, KY, May 2002.

154. American Academy of Pediatrics. Newborn screening fact sheet. (RE9362) 1996;98(3):473–501.

155. Newborn screening manual. Columbia, SC: South Carolina Department of Health and Environmental Control, 2000:43pp.

156. Sherwin J. Genetic testing in the state of California. PMF Monitor 2001(1):2–6.

157. Alcock GS, Liley H. Internet review article. Dr H Liley, Kevin Ryan Centre, Mater Mothers Hospital, South Brisbane, Queensland, AUSTRALIA.

158. Millington DS, Terada N, Kodo K, Chace DH. A review: carnitine and acylcarnitine analysis in the diagnosis of metabolic diseases: advantages of tandem mass spectrometry. In: Matsumoto I, ed. Advances in chemical diagnosis and treatment of metabolic disorders, Vol 1. New York: John Wiley and Sons, 1992:59–71.

159. Charrow J,Goodman S, McCabe ERG, Rinaldo P. Tandem mass spectrometry in newborn screening: American College of Medical Genetics/American Society of Human Genetics Test and Technology Transfer Committee Working Group. Genet Med 2000;2(4):267.

160. Chace DH, Millington DS, Terada N, Kahler SG, Roe CR, Hofman LF. Rapid diagnosis of phenylketonuria by quantitative analysis for phenylalanine and tyrosine in neonatal blood spots by tandem mass spectrometry. Clin Chem 1993;39:66–71.

161. Chace DH, Hillman SL, Millington DS, Kahler SG, Roe CR, Naylor EW. Rapid diagnosis of maple syrup urine disease in blood spots from newborns by tandem mass spectrometry. Clin Chem 1995;41:62–68.

162. Chace DH, Hillman SL, Millington DS, Kahler SG, Adam BW, Levy HL. Rapid diagnosis of homocystinuria and other hypermethioninemias from newborns' blood spots by tandem mass spectrometry. Clin Chem 1996;42:349–355.

163. Van Hove JL, Zhang W, Kahler SG, Roe CR, Chen YT, Terada N, et al. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency: diagnosis by acylcarnitine analysis in blood. Am J Hum Genet 1993;52;958–966.

164. Chace DH, Hillman SL, Van Hove JLK, Naylor EW. Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry. Clin Chem 1997;43;2106–2113.

165. Gibson KM, Bennett MJ, Naylor EW, Morton DH. 3-Methylcrotonyl-coenzyme A carboxylase deficiency in Amish/Mennonite adults identified by detection of increased acylcarnitines in blood spots of their children. J Pediatr 1998;132;519–523.

Acknowledgments

Section III

Dr. Rhone has received and Dr. von Dadelszen receives salary support from the BC Women's Hospital and Health Centre Foundation. Dr. von Dadelszen also receives salary support and establishment funding from the BC Research Institute for Children's and Women's Health. The authors gratefully acknowledge this support.

Section X

The following have shared their procedures to help create the guidelines in Section X:
Donald Chace, PhD, Neo Gen Screening, Bridgeville PA
David Millington, PhD, Duke University Medical Center, Raleigh NC
Steve Goodman, MD, University of Colorado Health Science Center, Denver CO
Rodney Pollitt, PhD, Sheffield Children's Hospital, UK
Kevin Carpenter, PhD, New South Wales Biochemical Genetics Service, Westmead, Australia
Mike Gibson, PhD, Oregon Health Science University, Portland OR
Piero Rinaldo, MD, PhD, Mayo Clinic, Rochester MN
Larry Sweetman, PhD, Baylor Research Institute, Dallas TX
.

Appendix A

A1. Letter of Comment from American College of Medical Genetics Laboratory

February 7, 2006

John E. Sherwin, Ph.D.
Chair, Genetic Disease Laboratory
State of California
Berkeley, CA 94710

Re: Draft Guidelines, Maternal and Fetal Health Risk Assessment

Dear Dr. Sherwin:

On behalf of the American College of Medical Genetics Laboratory Quality Assurance Committee, we are submitting these comments on the draft guidelines for Maternal and Fetal Health Risk Assessment put forward through the National Academy of Clinical Biochemistry. Specifically we are commenting on Chapters 4 and 5, which address maternal serum screening practices in the first and second trimesters of pregnancy.

The Laboratory Quality Assurance Committee is charged with writing and maintaining the ACMG Laboratory Standards and Guidelines for Clinical Genetics Laboratories. In this capacity we try to stay abreast of genetic testing guidelines put forward by other professional organizations. Your monograph provides informative examples of specific maternal serum screening programs and practices. We would like to comment on the following statements.

Chapter 4 – First trimester prenatal screening and diagnostic evaluation

1. "All screening programmers need access to a computer program that integrates maternal age, ethnicity, and smoking status with gestational age, ultrasound, and biochemical findings to give a modified age-related risk."

   We recommend that the list of factors to be included in the Down syndrome risk calculation be modified to include maternal weight. Additionally, we note that it is not standard for laboratories in the United States to include ethnicity or smoking status in the risk calculation. In the case of ethnicity, this may be due to a lack of consensus in the literature. We recommend that ethnicity and smoking status be removed from this list. Perhaps ethnicity and smoking status could be addressed by indicating that labs may further enhance their risk calculations by including these factors, but that such inclusion is not considered standard of care.

2. "Recommendation…That integrated age-based, nuchal translucency and biochemical screening be used to detect aneuploidy."

   A myriad of schemes for combining markers in the first and second trimester are being proposed. The term "Integrated screening" has come to have a specific meaning, referring to the scheme of combining first and second trimester markers, which was proposed by Nicholas Wald et al. (NEJM 1999;341:461–467). To avoid confusion, we recommend using the term "integrated" only in this specific context. Likewise, the terms combined, sequential, and step-wise screening appear to be taking on specific meanings and should be used with caution.

1. "Accuracy should be with 3% from lot to lot."

   This recommendation is both reasonable and necessary for the California State screening program. In this program testing is performed in several labs and those labs are all using the same medians and therefore need to stay in sync with each other. However, most laboratories are not part of a larger network of labs and are using medians specific to their own individual laboratory. Such stand-alone labs may not need to adhere to such a strict requirement. If necessary, a lab can adjust their medians to accommodate a large lot to lot change. Rather than recommend such a strict rule, we suggest offering laboratories more general guidance regarding the evaluation of lot to lot differences, establishing acceptance criteria, and decision points for changing medians.

2. "Repeat testing of initial positive results should not be performed."

   We recommend that this statement be clarified to refer to screen positive results for Down syndrome and Trisomy 18. Many programs do recommend repeat testing for minimal to moderate elevations of MS-AFP. We believe that this practice is acceptable, provided the patient's gestational age is early enough to allow for appropriate follow up.

We hope that you find these comments useful. If you have any questions, please do not hesitate to contact us.

Sincerely,

Michael S. Watson, PhD, FACMG
Executive Director

cc: C. Sue Richards, PhD, Chair Laboratory Quality Assurance Committee

A2. Response by Dr. John Sherwin

The editor received the attached comments from the American College of Medical Genetics recently, but significantly beyond the publication deadline. We have included these comments as an appendix since we want to include as much feedback from our colleagues as possible. We agree that maternal weight is a significant factor that should be included in the Down syndrome risk calculation. Experience from the California program indicates that ethnicity is an important factor in the risk calculation. We also appreciate that self-reporting of ethnicity is difficult to standardize. Nonetheless we encourage screening programs and others to continue to seek ways in which to include this information in the calculation. We would agree that the inclusion of smoking in a classic triple marker progrram may not be necessary, but programs using the quadruple marker calculation including inhibin should be aware that inhibin results are significantly affected by smoking. Once again, we recognize the difficulties inherent in including a self-reported variable. We recognize that during the period that this LMPG has been under review, the term “integrated” has taken on a different usage than its usage in this document. We accept that the term “combined” is probably a better term to use at this point in time.

We remain convinced that if programs demand this 3% level of precision from manufacturers they will get it. Further we are convinced that those programs that are not part of a larger group are probably the most at risk if precision and accuracy are not tightly controlled. Typically these are the programs that have the most difficulties with confirmation of their medians when new lots are placed in use. We need to continue to seek the aid of our colleagues in manufacturing to help us improve. Further we are concerned that repeat testing results in regression to the mean and has the potential to falsely classifying women. While many programs may recommend this practice, that does not make it appropriate for inclusion as part of the guideline.

In conclusion, we appreciate the comments from the ACMG and are pleased to be able to include them as an appendix. It is important to keep in mind that we are trying to establish a guideline that helps improve practice rather than just accepts the current practice. It should be noted that the entire Maternal and Fetal Risk Assessment field of practice is undergoing rapid change. We anticipate that these guidelines will be revised as the practice evolves.

Appendix B

Corporate Sponsors

Development and publication of these guidelines were supported by grants from the American Association for Clinical Chemistry (AACC) and the following:

Contributing Corporate Sponsors
Perkin Elmer
Adeza

Participating Corporate Sponsors
DPC
MicroMass
Diagnostic Chemicals

Appendix C

Reviewers and Commentators

Darrell Adams, ANSYS Technologies, Lake Forest, CA
Phillip Brewer, Yale University, New Haven, CT
Ken Buechler, Biosite Diagnostics, San Diego, CA