**The National Academy of Clinical Biochemistry**
**Laboratory Medicine Practice Guidelines**

# Draft Guidelines

## MATERNAL AND FETAL HEALTH RISK ASSESSMENT

**Comments should be directed to John E. Sherwin at jsherwin@dhs.ca.gov**

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Chapter 1 Defining Principles in Prenatal Pregnancy Risk Assessment and Reference Values

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Chapter 2 Vaccinations and Serologic Tests During Pregnancy

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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 the infection 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. (1)

**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 be immune 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. (1-5) 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 unimmunized or partially immunized should complete the primary series. Immunization of the pregnant woman with tetanus toxoid at least 6 weeks before delivery protects the newborn from tetanus neonatornm 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. (6)

**We recommend that pregnant women be immunized with diphtheria and tetanus toxoid (dT) so they are protected prior to delivery.**

Women in the second and third trimesters of pregnancy and the early puerperium are at increased risk of complications and hospitalization from influenza. (7) 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. (8)

**We recommend that pregnant women who will be beyond 14 weeks of pregnancy during the influenza season be vaccinated with the influenza vaccine.**

**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 polysaceharide vaccine.

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. (9,10)

Hepatitis A and hepatitis B vaccines, if indicated, can be administered to a pregnant woman. (1-5) 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 compared to adults. Vertical transmission orhepatitis B occurs in infants born to HBsAg-positive mothers with a 90% risk of developing a chronic infection without intervention. Preexposure immunization of susceptible individuals is the most effective means to prevent hepatitis B virus transmission. Risk factors that might indicate hepatitis B immunization of a pregnant woman include injection drug use, multiple sex partners, ajob that exposes one to blood or body fluids, living with someone who is infected, or having sex with someone whom 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. Postexposure immunization with HAV vaccine is recommended in adults. Although pregnant women safety data is 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. 1-lowever, 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.

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 9 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 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. (11)

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. (1) Women of childbearing age should wait at least 3 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.
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. (12)

S. pneumoniae is the most common cause of invasive bacterial infection and otitis media in children less than *5* 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. **(13)**

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 nontreponemal test (VDRL or RPR) and again at delivery. **(14) In** areas of high prevalence and in patients at high risk for syphilis, an additional nontreponemal serum test should be performed at the beginning of the third trimester of pregnancy (week 28). During pregnancy, low-titer false positive nontreponemal antibody tests may

occur. The nontreponemal antibody test should be confirmed as false positive with a treponemal antibody test (FTA-ABS). When a pregnant woman has a reactive nontreponemal test result and a persistently negative treponemal test result, a false positive test is confirmed.

**We recommend that all pregnant women be screened for syphilis early in pregnancy.**

**Rubella.** Postpubertal women without documentation of presumptive evidence of rubella immunity should be immunized, unless they are pregnant. (15) Postpubertal females should be advised not to become pregnant for 3 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.

**We recommend routine prenatal screening for rubella immunity, and rubella vaccine administration to susceptible women 3 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 postexposure immunoprophylaxis beginning at birth to prevent perinatal hepatitis B viral infection. (16) In high-risk individuals, repeat testing may be indicated in the third trimester. Postexposure 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.

**We recommend serologic testing of all pregnant women for hepatitis B by the hepatitis B surface antigen test.**

**Hepatitis C virus.** Seroprevalence among pregnant women in the United States is estimated at 1 -2%. Maternal-neonatal transmission is estimated at *5%.* Maternal coinfection with human immunodeficiency virus (HIV) has been associated with an increased risk of perinatal transmission of HCV (6-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. (17) Most infected women are asymptomatic and unaware of their infection. The 2 major tests for the laboratory diagnosis of IICV infection are antibody assays for anti-HCV and assays to detect HCV nucleic acid (RNA). Diagnosis by antibody assays involves an initial screening enzyme immunoassay (ELA). Repeated positive results are confirmed by a recombinant immunoblot assay (RIBA). Both assays detect IgG antibodies-no 1gM assays are available. PC'R 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 is definitely warranted.

**We recommend serologic testing of pregnant women for hepatitis C if clinically indicated or requested by a screening enzyme immunoassay (EIA).**

**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 HI V-infected pregnant women. It is recommended that all pregnant women be offered counseling and testing with consent for HIV. **(18)** 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 by 6-12 weeks after infection. Infants born to HI

V-infected mothers have transpiacentally 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 HI V-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 2 separate samples are positive. Infection can be excluded if 2 HIV DNA PCR samples are negative performed beyond I month of age and or I sample was obtained at 4 months of age or older.

**We recommend that all pregnant women have their HIV status evaluated by an appropriate antibody test after informed consent. Counseling must be provided regarding the results of testing.**

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Chapter 3 Preconception Care Issues and Pregnancy Diagnosis

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Chapter 4 First Trimester Prenatal Screening and Diagnostic Evaluation

**First trimester prenatal screening and diagnostic evaluation.**

**Dr Stephanie Rhone[1], MD, RDMS, FRCSC and Peter von Dadelszen[2,3], MBChB, DPhil, FRCSC;**
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***Outline.***

This document will cover the utility of the 11-14 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 is the integrated clinical, ultrasound, and laboratory management of ectopic pregnancy.

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***Utility of the 11-14 week scan.***

*Confirmation of viability.* That there is a role for a late first trimester ultrasound scan to confirm viability is self-evident. This has an important impact on maternal (and co-parental) wellbeing 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 made at the limits of fetal viability (23-24wks). The optimal timing of interventions such as prenatal diagnosis (biochemical, ultrasound, and invasive) or postdates induction of labour 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 > 7-10 days at 18-20wks.

*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 6-9wk gestation, with the presence of a thick membrane between gestational sacs being present in multichorionic gestations. The lambda (λ) sign of a dichorionic pregnancy is best seen at 10-14wks. As gestational age increases the dichorionic membrane becomes thinner, making the lambda sign less reliable after 16wk.

Monochorionic pregnancies are characterised by the absence of a septum, the absence of a lambda sign. However as the lambda sign in dichorionic pregnancies may disappear after 16wk, 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.

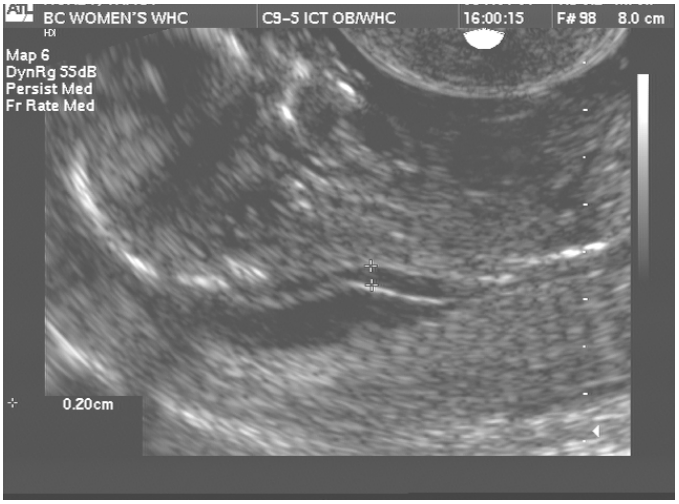
*Early diagnosis of major anomalies.* Reports of diagnosis of anomalies by first trimester ultrasound include such defects as those of the central nervous system (CNS; acrania/anencephaly), abdominal wall (omphalocoele), urinary tract (megacystitis), skeleton (caudal agenesis), and cardiac.

***First trimester screening for chromosomal anomalies.***

*Nuchal translucency.* 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.

*The standard for NT.* There is a set standard for NT for which certified training is required. The scan is performed at $11^{+3}$-$13^{+6}$wk (crown-rump length (CRL) 45-84mm), by transvaginal (TV) or transabdominal (TA) ultrasound scan. The fetal position should be one of neutral flexion, and $\geq$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" (Figures 1 and 2).



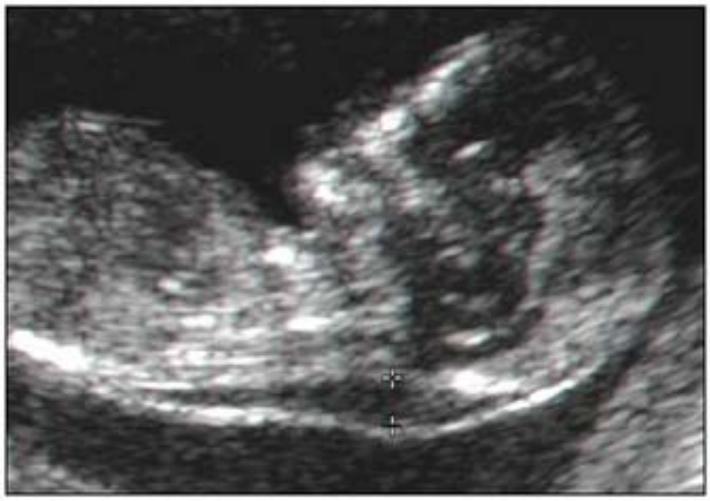



***Figure 1***. Normal fetus, NT 2.0mm.

***Figure 2.*** Trisomy 21 (12 wk, NT 3.5mm.

*NT Certification.* This is undertaken through a three-stage process. First a theoretical course (one day course and MCQ exam), then practical training with a log book of 50 images, and completed by a 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 monthly audit that includes a qualitative assessment of images and a quantitative assessment of the distribution of measurements within the site database.

*Increased NT and aneupoloidy*. 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 (Figure 3).

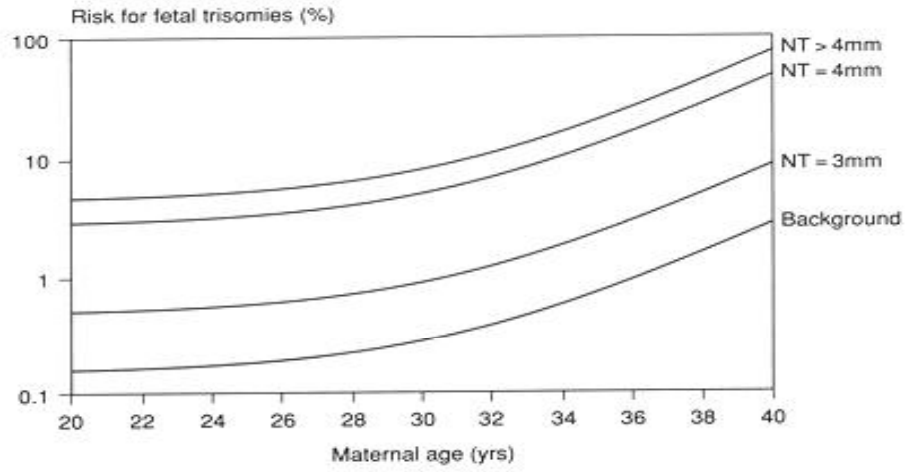



***Figure 3.*** NT and risk for trisomy by maternal age (Pandya P, *et al. Br J Obstet Gynaecol* 1995;102:957-62).

*Biochemical markers in the first trimester.* The goal of including biochemical markers in the first trimester 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 aneupolid fetuses without markedly increasing the false positive rate. The markers integrated in this approach are PAPP-A (Pregnancy-associated plasma protein-A) and free □hCG. This approach accounts for the impact of gestational age as PAPP-A increases and free □hCG decreases with gestational age (Krantz DA, *et al. Am J Obstet Gynecol* 1996;17:612-6).

*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 (that accepted for age-based screening, which remains the gold standard across North America), Gilbert *et al* (*BMJ* 2001:323:423-5) concluded that an integrated 1$^{st}$ and 2$^{nd}$ trimester was the most cost-effective approach, but may have limited acceptability for women who wish to conclude their prenatal diagnosis as early as possible and to clinicians who may be asked to withhold an abnormal NT result pending biochemical results at 15wks (Table 1).

***Table 1.*** The theoretical ('detection rate') and field ('reported rate') of screening for trisomy 21, using a 5% false positive rate (Gilbert RA, *et al*. *BMJ* 2001:323:423-5).

| 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 (□FP, hCG)** | 60% | 58-59% | 80% | 1 |
| **Triple test (□FP, hCG, $uE_3$)** | 68% | 67-69% | 80% | 1 |
| **Quadruple test (□FP, hCG, $uE_3$, inhibin A)** | 79% | 76-79% | 80% | 1 |
| **Integrated test ($^{11+15}/_{40}$) (1st TM: NT, PAPP-A; 2nd TM: quadruple test)** | 95% | 94% | 80% | 1 |

*NT, 1st trimester biochemistry, and other trisomies* In addition to trisomy 21 (Down's syndrome), NT and integrated biochemistry is effective in screening for both the other most common trisomies, trisomies 13 and 18. Setting screen positive limits at NT >3.3 MOM, PAPP-A <0.18 MOM, and free □hCG <0.28 MOM, will detect 89% of trisomy 18-affected pregnancies (Tul N, *et al. Prenat Diagn* 1999;19:1035-42). For trisomy 13, NT >2.9 MOM, PAPP-A <0.25 MOM, and free □hCG <0.51 MOM, will lead to a detection rate of 90% (Spencer K, *et al. Prenat Diagn* 2000;20:411-6).

*What is the impact of 1st 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 fetus detected. By employing an integrated 1st 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. It is the authors' belief that this approach should become the new standard of care.

*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. 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 >5.5mm (Hyett J, *et al. BMJ* 1999;318:81-5).
Genetic syndromes associated with an increased NT include Noonan syndrome, VACTERL, Zellweger syndrome, Joubert syndrome, Meckel-Gruber syndrome, and Nance-Sweeny syndrome.
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 (Souka AP, *et al. Ultrasound Obstet Gynecol* 1998;11:391-400). Souka *et al* found that the rate of healthy livebirth among fetuses with an NT 3.0-4.4mm was 90%, with an NT 4.5-6.4mm was 80%, and with an NT ≥6.5mm was 45%.

*The fetal nasal bone – the next refinement of the 11-14wk scan?* In an observational study, Cicero *et al* (*Lancet* 2001;358:1665-7) found that the absence of nasal bone was detected in fetuses with trisomy 21 at 11-14wks of gestation. This observation requires confirmation in appropriately powered series in other centres.

*The argument for making 1st 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. The evidence exists that NT integrated with either 1st or 2nd 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.

However, any screening programme 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 programmes need access to a computer programme 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 1st trimester clinic which coordinates the care of women presenting with threatened, inevitable, incomplete, and missed miscarriages (Wren J & Craven B. *Clin Perform Qual Health Care* 1999;7:172-7), possible or confirmed ectopic pregnancy (Luciano AA, *et al. Ann NY Acad Sci* 2002;943:235-54), and other 1st trimester complications. Such integrated clinics that utilise clinical, ultrasound, and laboratory modalities are recommended by both the Confidential Enquiries into Maternal Deaths in the UK, the UK Royal College of Obstericians and Gynaecologists, and American experts (Lipscomb GH, *et al. N Engl J Med* 2000;343:1325-9; Luciano AA, *et al. Ann NY Acad Sci* 2002;943:235-54). Early pregnancy assessment clinics have proven themselves to be cost-effective (Wren J & Craven B. *Clin Perform Qual Health Care* 1999;7:172-7).

***Ectopic pregnancy.***

The management of ectopic pregnancy has improved incalculably since the simultaneous development of trnasvaginal ultrasound and rapid quantitative □hCG measurement (Lipscomb GH, *et al. N Engl J Med* 2000;343:1325-9; Luciano AA, *et al. Ann NY Acad Sci* 2002;943:235-54). The concentration of □hCG ([□hCG]) should rise by >66% every 2d in a viable intrauterine pregnancy. Once [□hCG] >1,500IU/L an intrauterine fetal pole should be clearly visible transvaginally. Similarly, [□hCG] > 15,000IU/L should be associated with detectable fetal cardiac motion. The [□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 seen, then pseudodecidualisation 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 (folate antagonist, lethal to chorionic tissue), which is indicated where the woman is haemodynamically stable, there is no intrauterine pregnancy detected by ultrasound, the

ectopic <4cm diameter, and there is no evidence of rupture. Relative contraindications to methotrexate include visible fetal heart activity and a [□hCG] >10,000mIU/ml.
Before methotrexate, the following investigations should be performed: CBC, renal and liver function tests. Methotrexate is adminstered at 50mg/$m^2$ body surface area intramuscularly as a single dose. Following methotrexate the [□hCG] may rise over the 1st 3 days, but by day 7 there should be a minimum of >15% fall in [□hCG]. If not, a repeat dose of methotrexate should be given following CBC, renal and liver function tests. Once a response is noted, repeat [□hCG] are performed weekly until negative. Using this approach, in subsequent pregnancies 87% are intrauterine, and only 13% are repeat ectopics following a first ectopic.

Again, experts advise that this approach should be included within an integrated clinic, with established protocols (Lipscomb GH, *et al. N Engl J Med* 2000;343:1325-9; Luciano AA, *et al. Ann NY Acad Sci* 2002;943:235-54). In this setting, cost savings ($US3000 per treated patient), decreased morbidity, and improved patient satisfaction can be achieved.

***Recommendations.***

**That hospitals providing obstetric and/or gynaecological services develop Early Pregnancy Assessment Clinics to streamline the care of women with diagnostic issues in the 1st trimester. These clinics should link obstetric, ultrasound, and laboratory services.**
**That maternal age-based screening 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.**
**That integrated age-based, nuchal translucency and biochemical screening be used to detect aneuploidy. Until the results of the randomised controlled trials currently being conducted are known, units will need to decide for themselves on the balance of evidence whether they offer an integrated 1st trimester screen or a two-step 1st (Nuchal translucency, PAPP-A and □hCG) and 2nd (quadruple biochemistry) trimester screen.**
**That a fetus with an abnormal nuchal translucency, but found to be diploid, be offered fetal echocardiography.**
**That a fetus with an abnormal nuchal translucency, but found to be diploid and with a normal detailed ultrasound anatomical screen, be considered at increased risk for adverse outcomes, and be subjected to increased fetal surveillance for the remainder of the pregnancy.**

**That nuchal translucency be introduced only after appropriate training and certification, with access to the integrated computer programmes, and ongoing quality assurance.**
**That the safe and effective medical management of ectopic pregnancy is predicated on the co-ordinated efforts of obstetric, ultrasound, and laboratory services.**

***Acknowldegments.***

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Chapter 5 Second Trimester Prenatal Screening; Results from a Large Screening Program

**John Sherwin, Ph.D., Bob Currier, Ph.D., Fred Lorey, Ph.D. Chief, Genetic Disease Laboratory, State of California, Berkeley, CA 94710**

Introduction, History and General Description of the California Expanded AFP Screening Program.

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Chapter 5 Second Trimester Prenatal Screening; Results from a Large Screening Program

**John Sherwin, Ph.D., Bob Currier, Ph.D., Fred Lorey, Ph.D. Chief, Genetic Disease Laboratory, State of California, Berkeley, CA 94710**

Introduction, 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. Over 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. 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, over 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 are required to 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 will be paid by insurance or by MediCal.

The analysis is performed at one of eight regional laboratories[i] 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. The 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. Daily results are monitored using prepared internal quality control materials at 8 different concentrations, as well as by monitoring patient medians, analytical tray medians and periodic external proficiency testing. Results that pass Q/A 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. 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.

Screening for neural tube defects [and abdominal wall defects] is based on fixed cut-offs of the AFP MoM of 2.5 for singleton pregnancies and 4.5 for multiple gestations. This choice of cut-off 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. 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 consider positive if the risk is greater than or equal to 1:190 at midtrimester. This choice of risk cut-off 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, 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.

The central laboratory [GDL] is also responsible for evaluation of new lots of reagents. New kit lots are compared with the existing kit lot prior to use. This comparison is done using both quality control material as 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 I 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 in order that the new assay can be interpreted with appropriate medians, *i.e.* medians derived from that assay itself (Table 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 both an increased false positive rate and a decreased detection rate.

Monitoring medians and positive rates (Figures 1 and 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. 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 to help identify causes of variation of screen positive rates. One significant source of variation is ethnicity. We now adjust all three analytes for ethnicity—each in different ways—based on the 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.

The median MoMs represent the center of the population distribution, but the screening cut-offs 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.

**Recommendations**

**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 doing second trimester screening should consider adding inhibin A and/or invasive trophoblast antigen [ITA][1]**

[1] This was formerly known as hyperglycosylated hCG [HhCG].

Table 1. Validation of a new kit lot.

| Interpretation Version | Start Date | Change of Medians | **AFP Kit Lot** | Kit Start Date | Number of Samples | Gestation | | and We | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Median | log SD | Median | |
| 6.00 | 07/29/95 | X | 111111 | 7/29/1995 | 4399 | 1.03 | 0.1837 | 1.02 | |
| 6.00 | | | 222222 | 8/8/1995 | 51326 | 1.07 | 0.1830 | 1.05 | |
| 6.01 | 11/06/95 | | | | 25430 | 1.07 | 0.1793 | 1.05 | |
| 6.01 | | | 222222-plate | 12/21/1995 | 15458 | 1.03 | 0.1798 | 1.02 | |
| 6.10 | 01/18/96 | | | | 75787 | 1.03 | 0.1817 | 1.02 | |
| 6.10 | | | 222222-plate | 5/22/1996 | 12269 | 1.04 | 0.1797 | 1.02 | |
| 6.10 | | | 333333-std | 6/12/1996 | 6442 | 1.04 | 0.1849 | 1.03 | |
| 6.10 | | | 334333-tracer | 6/22/1996 | 9411 | 1.04 | 0.1817 | 1.03 | |
| 6.11 | 07/10/96 | | | | 33670 | 1.04 | 0.1868 | 1.03 | |
| 6.11 | | | 334333-plate | 9/9/1996 | 24498 | 1.05 | 0.1849 | 1.03 | |
| 6.11 | | | 334343-tracer | 10/23/1996 | 57534 | 1.02 | 0.1823 | 1.01 | |
| 7.00 | 02/07/97 | | | | 30887 | 1.02 | 0.1810 | 1.01 | |
| 7.00 | | | 334443-plate | 4/3/1997 | 7418 | 1.00 | 0.1777 | 0.99 | |
| 7.01 | 04/16/97 | | | | 62491 | 1.01 | 0.1823 | 0.99 | |
| 7.10 | 08/12/97 | | | | 26368 | 1.01 | 0.1843 | 1.00 | |
| 7.10 | | | 334543-plate | 10/2/1997 | 68304 | 1.05 | 0.1843 | 1.05 | |
| 7.10 | | | 444544-std | 2/19/1998 | 13393 | 1.06 | 0.1817 | 1.06 | |
| 7.10 | | | 444654-plate | 3/16/1998 | 31417 | 1.03 | 0.1791 | 1.03 | |
| 7.10 | | | 445664-tracer | 5/14/1998 | 28091 | 1.05 | 0.1792 | 1.05 | |
| 7.10 | | | **683741** | 7/9/1998 | 3222 | 1.07 | 0.1799 | 1.08 | |

Table 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% |

Figure 1. Monitoring assay medians and Down Syndrome screen positive rates Data are shown for last menstrual period dating.

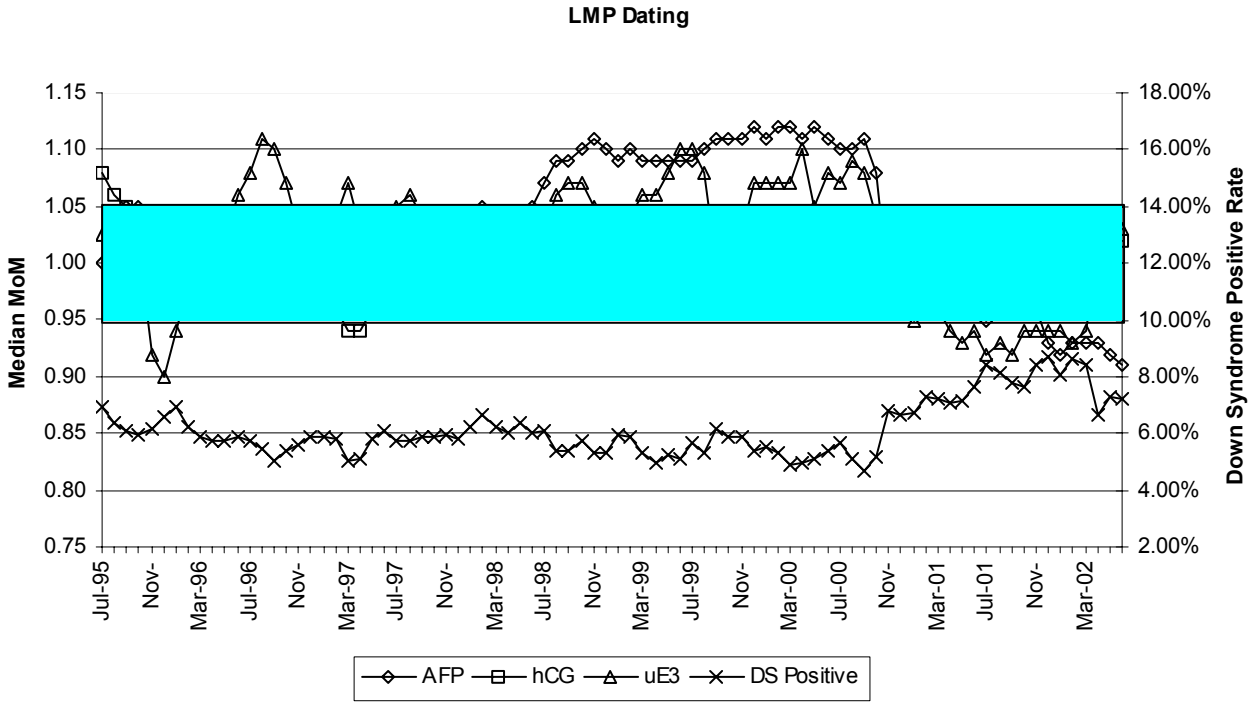
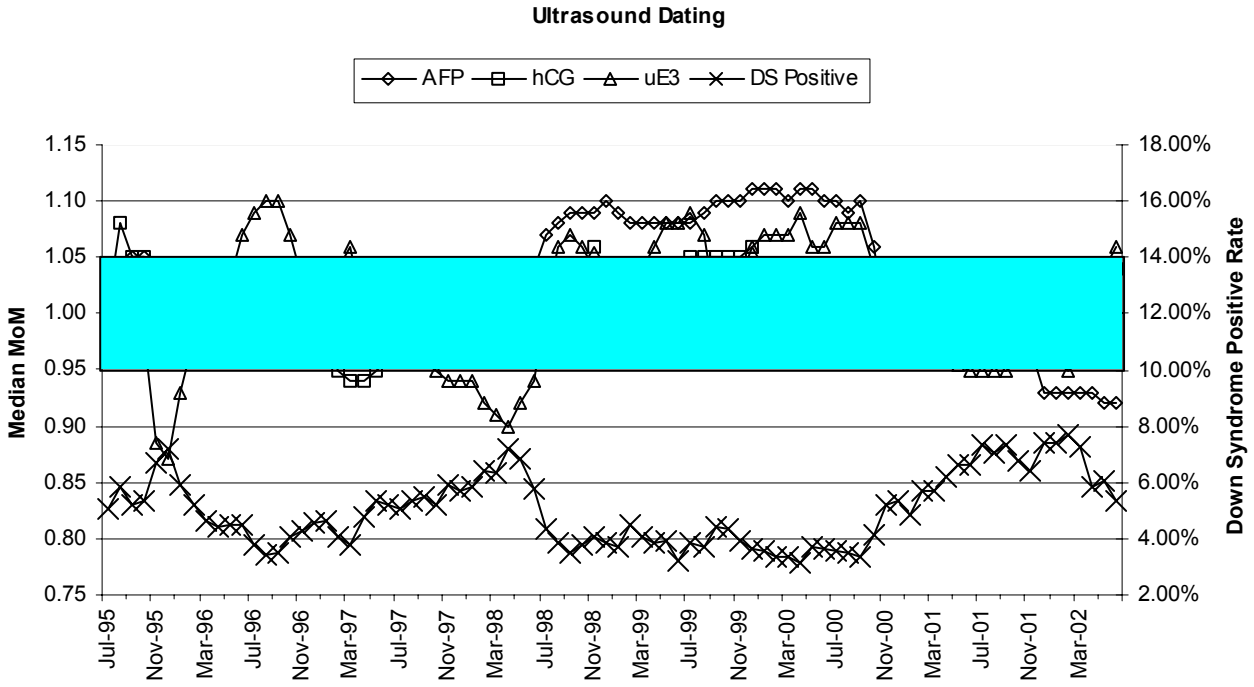


Figure 2. Monitoring assay medians and Down Syndrome screen positive rates Data are shown for ultrasound dating.



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Chapter 6 Follow-Up Diagnostic Assessment of the At-Risk Pregnancy

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## 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, the hypertensive disorders of pregnancy and thromboembolic disease for a number of reasons. Firstly, they are among the most common medical disorders of pregnancy. Secondly, they are the most serious of those disorders, being the most common causes of maternal death[ii]. Thirdly, the management of both is focused on laboratory testing. Finally, they 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. There is a lack of consensus in the literature regarding how one should diagnose and classify the hypertensive disorders of pregnancy (HDP), in addition to how one should manage them. This is due in large part to inconsistencies in terminology, for both the maternal HDP and the perinatal outcomes of interest. It 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 pre-eclampsia have been produced by the Canadian Hypertension Society (CHS)[iii], the US National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy[iv], and the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP)[v] (**Table 1**), 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[vi]. Therefore, in practice, the diagnosis of pre-eclampsia needs to be considered and excluded (by renal, hepatic, and haematological 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) arise. Furthermore, in a secondary analysis of the National Institute for Child Health and Development (NICHD) aspirin trial for the prevention of pre-eclampsia, women who developed severe non-proteinuric gestational hypertension (vs. those who developed mild pre-eclampsia) had higher rates of both preterm delivery (<37wk) and small-for-gestational-age infants[vii]. This reinforces the importance of considering the condition as other than one of pure hypertension and proteinuria.

Second, dichotomising pre-eclampsia 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, that gestational age has not been accounted for in any of the current classification systems is a major problem. It is the most important predictor of both maternal and perinatal outcomes. Early-onset pre-eclampsia (<32wk) is associated with a 20-fold higher risk of maternal mortality compared with pre-eclampsia that occurs at term[viii], and is consistent with more perturbed neutrophil function and cytokine levels. Also, gestational age is the most important determinant of perinatal outcome among diploid fetuses[ix]. A greater than 50% chance of intact fetal survival in pre-eclampsia arises only when the delivery gestational age is $\geq 27^{+0}$wk at a birthweight $\geq$600g[x].

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

**Predicting pre-eclampsia**

It is obvious that the current classifications of HDP are focused on diagnosing pre-eclampsia, 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.

Pre-eclampsia is a multisystem disorder which has its roots in inadequate placentation (e.g., having an immunological basis) and/or excessive fetal demands (e.g., multiple gestation). However, this mismatch is generated, there has been documented release from the utero-placental circulation, an intravillous soup. This includes various inflammatory mediator cytokines and trophoblast fragments that produce maternal systemic inflammation and the well-documented endothelial cell dysfunction which is thought to lead to 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 pre-eclampsia, by including 'adverse features' in the classification of HDP.

**Proteinuria as an essential component of the classification of HDP**

In the classification of HDP, proteinuria is key, but is 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 these can be improved with use of an automated device[xi]. 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, how to manage that woman 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 can be reported to the clinician on the same day. With a cut-off of >30mg protein/mmol of creatinine, the PPV is at least 90%[xii].

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, the 'gold standard'[xiii]. 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 pre-eclampsia.

**Recommended laboratory tests**

**Table 2 lists the recommended laboratory tests for the diagnosis and evaluation of pre-eclampsia. This list represents 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 hypoalbumenemia and the risk of pulmonary edema.**

**We surveyed Canadian practitioners, and asked them whether or not they use the commonly recommended tests in Table 2, and if so, how frequently they use them[xiv]. Most reported using all of the blood tests and the urine tests at least once weekly. The exception was urinary dipstick proteinuria which is used daily for women with suspected pre-eclampsia.**

**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 that we wish to avoid. This awaits further study, and until such time, the recommended tests in Table 2 are based heavily on expert opinion.**

**Differential diagnosis of pre-eclampsia**

The differential diagnosis of pre-eclampsia is that of underlying hypertension and/or other microangiopathies, such as thrombocytopenic purpura hemolytic-uremic syndrome, anti-phospholipid antibody syndrome, sepsis/disseminated intravascular coagulation, vasculitis, or malignant hypertension. Also, pre-eclampsia 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 pre-eclampsia must include further testing if the history and physical raise the suspicion of another disease process. Urinalysis may be particularly useful. In pre-eclampsia, 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 pre-eclampsia**

There are two issues to address: i) ruling out underlying conditions that may have predisposed a woman to pre-eclampsia, and may require or benefit from treatment (e.g., diabetes); and ii) identifying other cardiovascular risk factors because having had a HDP increases your long-term cardiovascular mortality and morbidity[xv].

Risk factors for pre-eclampsia

Risk factors for pre-eclampsia include pre-existing hypertension. Follow-up beyond six weeks postpartum is necessary, recognizing that the hypertension of pre-eclampsia may take a few months to resolve. Persisting hypertension should be regarded as pre-existing, and prompt investigation: electrolytes, creatinine, urinalysis, TSH, calcium, and a plasma renin:aldosterone ratio. Fasting blood glucose will detect underlying diabetes, and a follow-up 24-hour urinary protein (beyond three months postpartum) will detect persistent proteinuria suggestive of underlying renal disease. If pre-eclampsia was of early onset and severe, then thrombophilia testing is recommended, the details of which will be discussed below under 'Thromboembolic disease'.

Cardiovascular risk factors

After 6-12 weeks postpartum, whenat least the majority of the physiological changes of pregnancy resolved, it is appropriate to perform: testing for hyperlipidemia, hemoglobin A1C, hyperhomocysteinemia, as well as electrocardiography and echocardiography (to rule of left ventricular hypertrophy). For these women, as for women with gestational diabetes, pregnancy should be viewed as a 'stress test', which they failed, and which has afforded them the opportunity to appreciate their increased risk and 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) are associated with thrombophilia, either genetic or acquired. However, it has been more recently appreciated that thrombophilia may be related to adverse placentally mediated events: early severe pre-eclampsia; severe intrauterine growth restriction (usually defined as birth weight $<5^{th}$ or $<3^{rd}$ centiles), stillbirth, or recurrent fetal loss (defined as three or more consecutive, unexplained miscarriages)[xvi]. Although the association 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 screening with the advent of newer tests, such that testing reveals an abnormality in approximately 50% of individuals with thromboembolism or a family history of such. As shown in **Table 3**, 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, to date, problems with this haven't been described in pregnancy. 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, it must be recalled, that normal pregnancy is associated with an increase in procoagulant factors (e.g, Factor VIII), a decrease in some anticoagulant factors (e.g., Protein S) (**Table 3**).

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, and pre-eclampsia[xvii]. 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[xviii]. Other 'delivery' risk factors are: pelvic trauma, immobility and uterine sepsis.

Should all pregnant women be screened?

Screening is not advocated for pregnancy alone, just as it is not prior to taking the oral contraceptive pill, which is associated with a higher RR of thromboembolism than is pregnancy[xix].

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[xx]. If however, there is a family history of clot and/or there are one/more abnormalities on screening, then opinion favours 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, warrants treatment with low-dose aspirin (81mg/d) and low-dose heparin (unfractionated or low molecular weight)[xxi]. 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. Prophylaxis is agreed upon for symptomatic thrombophilia, especially multiple thrombophilias, symptomatic being maternal events, and antiphospholipid antibody syndrome specifically, and recurrent fetal loss specifically. Other women should be enrolled in studies.

Chapter 7 Evaluation of the High Risk Pregnancy at Term

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Chapter 8: Current Practices and Guidelines for evaluation of the newborn infant

**Barbara Goldsmith. (Ed. By C R Lee.)**

Children aren't just small adults….

The 1997 version of the NACB guidelines devoted to the special issues surrounding the newborn 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; as well as a discussion on detection of illicit drugs. Many of These recommendations, still stand today,

In the interim, many new point-of-care testing technologies have emerged. It is therefore worthwhile to include in the guidelines of 2003, the enhanced role of point of care testing within neonatal laboratory medicine.

With advances in modern medicine, the survival rate of newborns weighing less than 1000 grams has climbed; from about 0.5 percent, In the 1940s, to 5 to 10 percent by 1970 to current and by the 1990s, the survival rate in this population was over 60 percent. However, for the tiniest of infants, those weighing less than 500 grams, the survival rates remain about 1 in 16. (L. Lawson, "Technology and the Future of Neonatal Testing.")

Why does the neonate die? The causes most frequently associated with neonatal death 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 less than 1000 grams. Such infants often require respiratory support, circulatory support, and many have also undergone major emergency surgery.

Preterm birth rates are rising significantly. Preterm, defined as less than 37 weeks gestation, is the second leading cause of neonatal mortality in the United States. In 1999, in the US the prevalence of preterm births was cited as 8 – 12%, and a MMWR publication stated that black infants were about 1.8 times more likely to be preterm than white infants. From 1989 to 1996, while the overall rate of preterm births per 1000 live born infants increased 4 percent, the rate of multiple births increased 19 percent, thanks in part to increased utilization of fertility enhancement programs. Certainly, Multiple births are directly correlated with preterm births.

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. In the newborn, both fat content and water content differ from values seen in older infants, Water content in a full term infant my 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 liver function can be affected due to hepatic immaturity, causing a slower rate of metabolism and excretion of drugs. And infants with immature organs who receive drugs are very susceptible to drug toxicity because their immature organs can't handle both the metabolism and the excretion of those drugs.

**Age specific reference ranges.**

This is a challenge for pediatrics in general and for neonates in particular. Age-specific reference intervals are critical for appropriate interpretation of 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 [13,14] 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 adult reference intervals are used We recommend providing age-related gestational and postnatal reference ranges where available

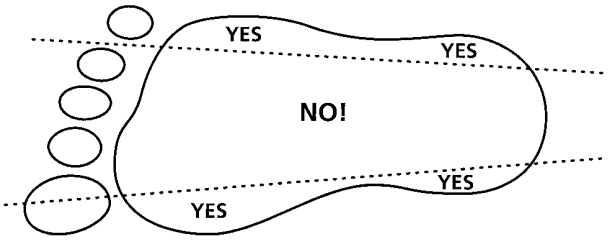
Table 1 lists a typical comparison of common markers in the adult with those of a full term infant.

from STAT testing in the neonate" by Barbara Goldsmith, Ph.D. "Blood Gas News", vol 11, No. 1. *Note: reference intervals will vary, depending on instrument and method used.*

| Analyte | Adult range | Full term newborn |
|---|---|---|
| Albumin | 3.7 – 5.6 mg/dL | 2.6 – 3.6 mg/dL |
| Total protein | 6.3 – 8.5 mg/dL | 3.4 – 7.0 mg/dL |
| Alkaline phosphatase | 50 – 175 IU/L | 50 – 400 IU/L |
| Ionized calcium | 4.8 – 5.3 mg/dL | 4.8 – 6.0 mg/dL |
| Uric Acid | 1.8 – 5.0 mg/dL | 3.0 – 8.6 mg/dL |
| Thyroxine | 4.9 – 10.0 μg/dL | 5.9 – 21.5 μg/dL |

| | | |
|---|---|---|
| Total Bilirubin | < 1 mg/dL | <12 mg/dL |
| CKmb% | <2.0 % | 1.5 – 8.0% |
| Phosphorus | 2.8 – 4.5 mg/dL | 4.5 – 8.0 mg/dL |
| Ammonia | < 35 μmol/L | < 50 μmol/L |

Alkaline phosphatase. infants are higher due to rapidly forming bone structure.
Thyroxine has an upper reference range of 21.5, 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.




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

Issues of Particular concern:

**Collection of specimens**. This is labor intensive, but critical to the management of NICU laboratories to have dedicated individuals well trained in collecting specimens on these tiny and ill infants

**Metabolic diseases.** Pediatric laboratories should have ready access to reference laboratories having equipment needed for analysis and monitoring of amino acids, chromatograms, organic acid analysis, 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 from there 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.

Heelstick.

The standard for practice for heelstick phlebotomy continues to be the NCCLS approved standard. This recommendation provides a map for proper placement of the lancet with respect to configuration of the infant's foot. (see. Fig X)

**We recommend that phlebotomists be trained on and follow the NCCLS document NCCLS – Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture: Approved Standard – 4 ed. (1999), H4-A4.**

Other Key points of this recommendation include:

**The optimum depth of the puncture.** The selection of the lancet should dictate a puncture depth of $\leq$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 approx. 10% higher with skin puncture for some analytes. Because the heels of infants in the NICU are frequently punctured, edema can result. This can cause contamination with tissue fluid, leading to an increase in certain analytes, particularly hemoglobin, potassium, and LD.

**Preanalytical concerns** may present more of a challenge **in** pediatrics. Many factors may influence results.

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, $CO_2$, Cl can increase by as much as 30% if spun without caps

(Young DS, Blood Gas News, 11: 14-18 2002)

**Capillary blood.** If capillary blood is used, it is important to note a couple of differences. Due to tissue metabolism, the pH is higher in capillary blood than in venous blood. Glucose can be also be about 0.5 millimoles per liter higher than plasma, and 0.4 millimoles higher than whole blood. TSH, TBG, and T4 are higher than in venous blood. Capillary tube blood for blood gases must be mixed, sealed, placed in ice water; pH can decrease by 0.005 every 10 minutes at room temperature. In order to achieve the turn-around time needed, they have to be analyzed very quickly and, optimally, either at the bedside, or in the NIC-U.

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

**Preanalytic concerns, in skin puncture**. Once the skin is punctured, the blood should flow freely as droplets into the collection tube, and if anticoagulant is present in the tube, ensure adequate mixing. Betadyne contamination causes increases in potassium phosphorus chloride, $CO_2$, and uric acid. Hemolysis is a frequently seen due 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.

**Interfering Substances in methods:** 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 present interferences in a variety of analytes. Interference on bilirubin with hemolysis is method dependent. The Jendrassik-Grof bilirubin procedure exhibits decreases in concentration due to hemolysis. Conversely, an increase in bilirubin is seen with the 2,5 dichlorophenyldiazonium detergent procedure.

We recommend that manufacturers should provide information concerning the impact of preanalytic factors concerning specific tests, and that laboratories should consider appending them to reports.

A recent study conducted (Griffith CH, Crit Care Medicine, 25:704-709 1997) in a very busy NICU looked at the test ordering patterns of the house staff of 8 residents and 6 interns. With data collected on 785 infants,

no differences were observed in x-ray and electrolyte ordering; but significantly more ABGs were ordered by interns than by more experienced residents. [5]

Specimen labeling.

This may be a challenge for the short blood collection containers that are frequently used. Particularly, the bullet tubes and the microspecimen containers are 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. (ie, Smith – Twin A, Smith - Twin B) It is important for the lab and nursery to agree on a naming convention for multiples that is compatible with the Lab information system.

**Specimen volume** represents a challenge for the laboratory Formerly, Instrumentation was a bigger concern for small sample sizes and precise pipetting of very small sample sizes. Today, most instrument vendors can accommodate the needed small sample sizes and container dead volumes.Hematocrits in newborns and neonates are frequently 60 percent or higher, which results in a smaller yield of plasma and/or serum. The blood volume of neonates can be estimated with nomograms (figure 7) factoring in age and size, to help assess how much blood volume 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 percent of babies of less than 1500 grams, 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 full term and 100 mL/kg for preterm infant. In recent years transfusions have decreased due to more transcutaneous monitoring, and new instrumentation requiring less blood. And with more both *in vivo* monitoring, and point-of-care testing, the need for transfusion and the requirement for excessive blood is predicted to decrease.

Urine specimens: . It is preferable to use random specimens or timed collections rather than 24-hour 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.

**It is recommended that the laboratory work with clinicians to ensure a proper urine specimen, if needed**.

**STAT and urgent specimens: Turn-around times for results.** Turn-around time is defined as the time interval from specimen collection to the receipt of results For most pediatric laboratories, it is not uncommon to see requirements for stat turn-around times on 50 to 60 percent of the specimens received, compared to 30 to 40 percent in an adult setting. A test that is performed off-site, even when performed on a device taking 10 minutes can have a TAT as long as a 30 minutes test which is performed in the lab or at bedside. In critically ill infants, analytes like electrolytes, blood glucose, and blood gases should have a TAT within minutes, with everything else ASAP. Critical tests should be available 24 hours per day. Some tests don't require availability 24 hours a day, and daily measurements should be adequate.

We recommend that the laboratory 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.).

Table #. Recommended Turnaround Times for Pediatric Analytes.

**Dead volume** is defined as the volume of specimen that cannot be sampled from the cup or sample container. Some analyzers are able to achieve very small dead volumes of 40 or 50 microliters, while the dead volume for other analyzers can be up to 250 microliters, depending on the allowed containers, even though the analyte itself might use only 2 to 3 microliters per test. Most modern instruments are capable of achieving precise pipetting of small specimen volumes

We recommend using narrow, deep containers for very small sample sizes in order to minimize specimen evaporation and to minimize container dead volume.

**Analytical range**. Because so many of these specimens are required on a stat basis, 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 milligrams per deciliter without the need for dilution.

**Fluid and electrolytes in neonates.** As mentioned previously, physiologic changes can be significant in the first couple of days, or 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 the skin, due to higher permeability, which can lead to dehydration, and abnormal electrolytes. The extracellular water loss may lead to weight loss of from 5 to 10 percent in a term infant, and as much as 10 to 20 percent in a preterm infant during the first week of life.
Close monitoring of electrolytes is required. Avoid reporting potassium on visibly hemolyzed specimens. a critical electrolyte result obtained from a from a skin puncture should be confirmed with that from a non-skin puncture, preferably from a line, if available, or from a venipuncture.

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 [I 5,16), and may be caused by low cardiac output, congenital heart disease, lung disease, anemia or hemoglobin variants [7]. 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 (1 7,181, The therapeutic goal is adequate delivery of oxygen without undue stress on the organs, such as the lung and retina.

Oxygenation, alveolar ventilation, and acid-base status must be monitored during the neonatal period when cardiac and /or respiratory dysfunctions occur. This can be done both at the bedside and in the laboratory. Blood gas measurements, often referred to as arterial blood gases (ABG), 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 [16,19,20]. Caution should be given to interpreting ABG values in patients with hyperbilirubinernia, anemia, or those receiving hyperalimentation. ABG specimens from patients with these conditions 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-S02(a) values 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 [21]. 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 (22). Pulse oximetry is less susceptible to this shift than CO-oximetry for monitoring $O_2$ saturation.

Newer technologies for measuring blood gases include continuous *in vivo* and *ex vivo* monitoring systems [23-25]. *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 [19]. Management of an increased $pCO_2$ may involve decreasing $C0_2$ production (e.g. sedation, reduction of thermal stress) or by increasing ventilation (e.g. -increasing 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 $C0_2$ ($tCPCO_2$) or end tidal $C0_2$ ($PCO_2$(ET)) monitoring. Though the $tcpCO_2$ method is preferred for preterm neonates, each device has limitations that require validation by ABG measurements.

The assessment of acid-base status is critical in the compromised neonate. The most common causes of metabolic and respiratory acidosis and alkalosis are listed in Table IV [32]. 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 [261. Blood obtained from indwelling catheters yields the most accurate measurement Of $P0_2$(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 hepa6n is recommended to avoid dilution effects [19,27]. 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 [9]. Capillary $pO_2$ measurements are unreliable in ill infants and not recommended [7]. See table IV.

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 [19,28], 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 turnaround t times and appropriate age-related reference intervals.

**Neonatal jaundice. This common condition is observed in 60 percent of the term and as much as 80 percent of preterm infants in the first week of life. It is the visual product of bilirubin deposits in the skin and mucous membranes. Physiologic jaundice is defined as 13 milligrams per deciliter, or 212 micromoles per liter, (SI units), in the first week of life.**

Bilirubin

Serum bilirubin determinations are frequently required for the routine management of the newborn. Bilirubin measurement 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 are 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 be derived from impaired albumin binding that can lead to increased bilirubin levels. There may not be enough binding sites on albumin for bilirubin, which releases bilirubin into the circulation.. An infant can be hypoalbuminemic, again, not providing enough binding sites for bilirubin. Concurrent acidosis can also lead to increased bilirubin as well as treatment with drugs that displace bilirubin from albumin.
Recent advances in neonatal therapy on anti-immunoglobulun postive infants indicates that treatment with intravenous immunoglobulin has been suggested as an alternative therapy for isoimmune haemolytic jaundice to reduce the need for exchange transfusion.

**Main results**

Seven studies were identified. Three of these fulfilled the inclusion criteria and included a total of 189 infants. Term and preterm infants and infants with rhesus and ABO incompatibility were included. The use of exchange transfusion decreased significantly in the immunoglobulin treated group (typical RR 0.28, 95% CI 0.17, 0.47; typical RD -0.37, 95% CI -0.49, -0.26; NNT 2.7). The mean number of exchange transfusions per infant was also significantly lower in the immunoglobulin treated group (WMD -0.52, 95% CI -0.70, -0.35). None of the studies assessed long-term outcomes.

**Reviewers' conclusions**

Although the results show a significant reduction in the need for exchange transfusion in those treated with intravenous immunoglobulin, the applicability of the results is limited. The number of studies and infants included is small and none of the three included studies was of high quality. The protocols of two of the studies mandated the use of early exchange transfusion, limiting the generalizability of the results. Further well-designed studies are needed before routine use of intravenous immunoglobulin can be recommended for the treatment of isoimmune haemolytic jaundice.
Reference: Alcock and Liley.

**We recommend that liver function be evaluated using a combination of bilirubin testing and liver function enzyme testing.**

Glucose in neonates.

Glucose is one analyte where high concentrations as well as low concentrations can be dangerous to the neonate. Neonates are at risk of hypoglycemia immediately after birth (34), with the risk increased in preterm neonates whose hepatic glycogen stores are low [351. Hyperglycemia, particularly in the preterm infant, may occur following glucose administration due to a sluggish insulin response [36]. Frequent monitoring is required in these patients, often performed using point-of care (POC) glucose monitors. Because of the higher hematocrit levels in neonates in general, and neonates receiving oxygen therapy in particular, devices and test strips must be evaluated and correlated to laboratory methods for appropriate interpretation of results [37,38). In addition, there is a difference of about 11 % between whole-blood POC glucose and 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, 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 of blood glucose levels is key. In unexpected hypoglycemia, the infant should be evaluated for inborn errors of metabolism. For assessment of hypoglycemia, there is no consensus for the cut off point. However most Many NICUs try to maintain concentrations between 3 mmol/L (>54 mg/dL) and 10 mmol/L (<180 mg/dL). Among neonatologists, Management of hypoglycemia can be modified with changes of 1 mmol/L or 18 mg/dL. Therefore accurate measurement and close monitoring is essential.

(Hawdon JM, Blood Gas News 11:37-40 2002)

**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 [39). Changes in creatinine vary with gestational age, and the absence of *an* expected drop may indicate compromised renal function [40

**Lactate.** Lactate can accumulate in tissues, blood and CSF from anaerobic metabolism often caused by crying. Measurements of lactose provide an indication of adequacy of recent or current oxygen delivery to the tissues, and can be essential in the diagnosis of inborn errors of metabolism. Small point-of-care systems for whole blood lactates are now available that can be located in a NICU setting virtually at the bedside.

**Therapeutic drugs**. Approximately 12 percent of all drugs prescribed in the U.S. are for children less than nine years of age. For premature infants less 1000 grams, the number of drugs given during hospitalization averages 15 to 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. And Due to the immaturity of enzymes pathways, and decreased protein binding, biotransformation of the drugs into metabolites and the bio-usable form is slower.

**Point-Of-Care Testing.** The small specimen requirements and the rapid turn-around time make point-of-care very well suited for the neonate. Many of these devices are capable of performing multiple analytes on a whole blood specimen of 100 microliters or less. This is less than the requirement to draw, send to the lab, spin down, and aspirate into the analytical device.

It is necessary to validate these point-of-care devices for neonates with respect to interferences typically seen and the differing range of concentrations seen from 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 40 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 very applicable to the neonatal population. Point-of-care Devices, fall in two groups: electronic-based point-of-care devices and non-electronic based testing.

The non-electronic have been is use for many years and include manual procedures, are indicator based and might include qualitative positive/negative answers like pregnancy tests or urine dipsticks that gives you semi-quantitative or semi-qualitative 1 plus, 2 plus, 3 plus types of results.
The electronic can be transportable, portable, hand-held formats, and can be either stationery, as in a NICU or go from patient to patient. Methods used for point-of-care include electrochemistry, reflectance photometry, and immunology-based methods. There are non-invasive technologies available for glucose and bilirubin.

The "ideal" point-of-care device is small, robust, lightweight, uses a small sample size, and is easily transported. Lockout features are very important. Some systems require passcodes, so that if an operator isn't trained, the test can't be performed. With QC lockout, the test can't be performed if QC isn't done. And if patient ID isn't entered, then the test is not performed.

Examples of POC analyzers currently used in the NICU setting include the SureStep Probe from LifeScan for glucose, The Hemocue from ------ for hemoglobin, the -- the ITC 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 the same as that from laboratory for a number of reasons. Glycolysis during transport can lower the result in the laboratory vs. the bedside results. Whole blood can be as much as 11 percent higher than plasma due to spin time and contact with red cells. 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. CVs typically seen the lab for glucose are 5 percent or less. FDA approval typically requires 20 percent. Preanalytic issues can also contribute to the differences as well as staff compliance and competency in performing the point-of-care tests.

**potential errors seen in neonates.** There are differences between whole blood values and values expected from plasma. The high hematocrits often seen in neonates (> 60%) can impact these measurements. Many devices convert from whole blood to plasma values, and 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.

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 as shown in Table X. below.

| 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 Sure Step | 190.6 | 116.7 | 74.7 |
| Medisense PCx | 164.6 | 96.3 | 55.2 |
| Roche Advantage | 154.7 | 88.6 | 45.6 |

Suggestions for avoiding potential errors in using blood glucose meters would include: (Tang Z. et al. Advance/Laboratory, August 2000)

- Know 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 technologies are emerging rapidly in point of care testing. 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.
A good example of an *in vivo* -- or *ex vivo* monitor with an *in vivo* line would be 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. Two studies published in *Pediatrics*, found a very good correlation with laboratory analyzers. (6, Weiss, et.al, and 7 Widness, et.al)

The BiliCheck, point-of-care bilirubin is non-invasive hand-held device that uses multi-wavelength spectral analysis to take a transcutaneous measurement on the baby's forehead. Bihutani, et.al 8. looked at 490 pre-discharge term and near-term racially diverse newborns. A matched sample comparison of heelstick measurement using a gold standard HPLC measurement vs. the Bilicheck from SpectRx, Inc, showed a very good correlation of r = 0.91 in the range of 0.2 to 18.2. Two other outcomes of the study were noted. One, skin color was not found to be a significant variable. Two, infants potentially at high risk for developing hyperbilirubinemia after being sent home within 48 hours were able to be identified..

The Cygnus GlucoWatch by Biographer is a minimally invasive device for monitoring glucose that is FDA approved. Glucose is extracted through the skin by reverse iontophoresis, and

using an applied electrical potential, and detected by electrochemical enzymatic sensor, three measurements per hour can be obtained. in one study by Tamada, 8.. more that 1,500 data points from 28 patients with Type I diabetes over 18 years old, were compared to a the Hemocue point-of-care analyzer with a high degree of correlation of results. R= 0.90. The black dots are the GlucoWatch and the triangles are the blood glucose point of care, blood glucose measurement, in this case done by the Hemocue. While there is an apparent bias in actual results, the measurements track very well.

Tamada JA et al, JAMA, 282: 1839-1844 1999

While this study was not done in a pediatric setting, the device is inherently suitable for pediatrics due to its non-invasive nature.

OPS, (orthogonal polarization spectroscopy) is another methodology with future potential. Using sublingual in vivo imaging, the flow rate of blood through the small capillaries in the tongue is measured by a probe placed under the tongue. It is possible to measure red blood cells and a partial CBC by this technique.

It is recommended that the laboratory consider the utilization of non-invasive point of care 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. However, the effect of interferences (e.g. high hematocrits) and differing concentration of analytes from adults must be validated on POCT devices.

**We recommend that these critical tests be done prior to the infant's leaving the hospital**

| | |
|---|---|
| **these tests may include:**<br>**Rubella (German Measles) Immunity** | **Newborn Screening Testing mandated by the Governing Body** |
| **HIV** | **Toxoplasmosis** |
| **Hepatitis B screen** | **RH antibody screen (citation #3)** |
| **Hemoglobin** | |
| **Hemoglobin abnormalities screen, based on family/medical history** | |

Once the baby is born, the testing performed will provide information needed to give the baby the healthiest start possible. If the results indicate a problem, treatment can often be administered before the baby is symptomatic.

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 rarely causing an infection. Each year infections develop in over 50,000 pregnancies. If an infection develops, the bacteria may infect the uterus, amniotic fluid, urinary tract, and any incision made during a cesarean section. During the birth process, the baby may be infected by inhalation or by ingestion of the bacteria. Approximately 8,000 babies in the United States contract serious GBS disease each year, with a 10% fatality rate and up to 20% of the babies who survive GBS-related meningitis are left permanently handicapped.

In newborns, GBS is the most common cause of sepsis (infection of the blood) and meningitis (infection of the fluid and lining surrounding the brain) 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. Some babies that survive, especially those who develop meningitis, may develop long-term medical problems, including hearing or vision loss, varying degrees of physical and learning disabilities, and cerebral palsy.

GBS is considered as a leading cause of serious neonatal infections and newborn deaths. In a Year 2000 report from The Centers for Disease Control and Prevention (CDC), it was estimated that of the more than 1600 GBS infected newborns in the United States, approximately 80 died. Schrag et al. concluded in their article of the July 25, 2002 issue of The New England Journal of Medicine, that routine screening for GBS prevents more cases of early-onset disease than the risk-based approach. In 1996, the Centers for Disease Control, the American College of Obstetrics and Gynecology, and the American Academy of Pediatrics issued aggressive guidelines for prenatal screening and prevention of GBS Disease. Vaccine development is still underway as the ultimate prevention.

Infected infants may display symptoms within six hours following birth or as late as two months of age. If untreated, the baby may develop a life-threatening systemic blood infection, pneumonia, hearing or vision loss, or varying degrees of physical and learning disabilities.

Infection in the neonate, particularly in the first day of life, is a life-threatening and relatively common clinical entity (estimated at 8 cases/1000 live births). However, 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 timeframe requisite 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 usually based on clinical impression.

Efforts to identify a sensitive and reliable laboratory parameter 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 hospitalization, subjection of newborns to intravenous therapy, and the drive for medical cost containment.

The acute phase proteins (fibrinogen, alpha1-antitrypsin, haptoglobin, ceruloplasmin, C-reactive protein, and alpha$_1$-acid glycoprotein) have been the subject of numerous investigations. The lagtime between onset of infection and production of acute-phase proteins accounts for disappointing sensitivity and positive predictive values for testing. Current practice (citation 2) suggests that a combination of CRP, interleukin-6, and procalcitonin testing in the early post natal period may detect infection in a higher risk asymptomatic infant with an infected mother.

Calcium and phosphorus

During the last trimester, calcium and phosphorus are incorporated into the bone matrix. Therefore, the preterm infant has greater needs for these two minerals than term infants. In Perenteral 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. Because of this inability to meet the increased demands for calcium and phosphorus relative to limitations with solubility, 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 by **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 [33).

We recommend that ionized calcium is the preferred method for testing of calcium.

Chapter 9 Newborn Metabolic screening

Each year 4 million infants in the U.S.A. and xxxxx worldwide are screened to detect conditions that threaten their life and long-term health. 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 it's territories and possessions. Each state is responsible for determining which tests should be performed on newborns, however health care 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. The implementation Involves 4 Agencies: HRSA (Health Resources and Services Administration) , AHRQ Agency for Healthcare Research and Quality), CDC, and the NIH. Worldwide, The Association of Public Health Laboratories comprises more than 250 laboratories in the United States and 45 other countries, as shown in yellow on the map in Fig 6. The Association of Public Health Laboratories partners with The CDC to provide services aimed at ensuing the quality of testing. These services include: Filter paper evaluation, Training, consultations, and Proficiency testing
Fig. 6

Table 3. Distribution of metabolic screening in the US.

Table of Cutoff points.
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 3 states offered a screen for the hemoglobinopathy causing sickle cell disease. Other metabolic screening tests are available and may be performed based on state requirement, family history, or to diagnose the cause of symptoms displayed by the baby. 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 from heelstick blood collection. Table #3 lists those conditions and tests which are performed in at lest one state testing program, and the methodologies by which testing is performed.

In California from 1980 through 1997, over 8.5 million infants were tested in the newborn screening program, detecting 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, over 4 million infants have been tested. From 1995 to December of 1997 nearly 1000 cases of sickle cell disease were identified and 131 cases of clinically significant hemoglobinopathies were referred for follow-up care. In 1997, The CA 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.

States conditions are screened in :

| | |
|---|---|
| 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 |
| Congential Adrenal hyperplasia | 18 |
| Toxoplasmosis | 2 |
| HIV | 1 |

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.

Methododologies

Screening tests are performed on dried-blood-spot specimens collected from newborns. Depending on the particular test, a wide variety of standard and state of the art methods are

used including colorimetric, immunoassay, radioimmunoassay, HPLC, flourometric, 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. The following table lists those disorders that have been documented to be detectable by MS/MS.

Congressional interest

In the US 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 Centers for Disease Control and Prevention and the National Institutes of Health, is 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 the 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 AAP (American Academy of Pediatrics) Task force included:

Use of 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 statewide advisory group.
Safeguard blood samples.
Provide adequate financing for testing, diagnosis, and treatment.

Testing for specific conditions detectable in the newborn

Congenital Hypothyroidism

*Congenital hypothyroidism* occurs when a malfunction in the development of the thyroid gland due to full or partial glandular development (aplasisa of hypoplasia) or an ectopic location, results in insufficient production . of thyroxine, the primary growth regulating hormone and one necessary for proper nervous sytem development., it's absence causes slow growth and mental retardation. Mental retardation can be avoided if detection and treatment with thyroid supplements occurs 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 US. (citation).

Testing performed:
Screening usually consists of measuring either Total Thyroxine (T4) or thyroid stimulating hormone (TSH) or both.
The cutoff point for Total T4 varies from xxxx to xxxxx depending on the program.
According to Jewell et.al., the greatest source of imprecision in either thyrotoxin or thyrotropin is the variation among manufacturer's kits at the low concentration which was within the range of cutoff values for detection of presumptive positive specimens.

Limitations of congenital hypothyroidism screen
Thyroid testing measures the amount of hormone that is present in the baby's blood at the time the test is performed. At birth, thyroid hormones from the mother are present in the baby's circulation that 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 less than 24 hours of age. 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 12 hours of age, a second specimen should be tested before two weeks of age.

**Premature infants:**

In some premature infants there has been noted a transient physiological effect manifesting as lowered 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 an autosomal recessive disorder where the body fails to produce the enzyme phenylalanine hydrolase, preventing the conversion of phenylalanine into tyrosine. Phenylalanine, an essential amino acid, is normally converted to tyrosine by this enzyme, which uses tetrahydrobiopterin as a cofactor. Normal metabolism of phenylalanine results in a serum concentration between 30μ M 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 results in developmental delays, seizures, acid odor, and severe mental retardation, if not detected and treated early. Restricting the diet of phenylalanine and monitoring the serum levels has 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.

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 falls to normal within 24 hours of birth. PKU mothers should maintain levels of PKU between 2 and 6 mg/dL in order to avoid damage to the developing fetus.

Limitation of PKU test

Collection of 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 result in 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, somewhat lower among other ethnic groups.

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 Tests:

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:

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.

| Abnormal Results | Likely Causes | Recommended Follow-up |
|---|---|---|
| IRT > or = to 90 ng/mL (CO/WY) > or = to 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 > or = to 70 ng/mL (CO/WY) > or = to 80 ng/mL (MT) | Cystic Fibrosis Early collection ofspecimen False positive | Diagnostic sweat testing |

Galactosemia

This test is performed in the majority of states. Babies who inherit this disorder cannot metabloize the sugar galactose found in milk, breast milk, formula and other foods. This results from an inability to break down the sugar galactose. Within the first two weeks of life, untreated infants born with this condition experience vomiting, liver disease, mental retardation, cataracts and failure to thrive.

**Providing a milk-free diet is the recommended treatment for galactosemia, and can help prevent these problems.**

Limitations of Galactosemia Screen
The test does not detect carriers. . For galactosemia, the most common cause of false positives has been heat denaturation of the enzyme during transport.
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.

Causes of false positives or negatives
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 1:60,000 births.
From the AAP Newborn screening fact sheet. Reference # 4.
*Type of Test.* Colorimetric assay (for biotinidase) 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 2 months of age, but one patient was symptomatic at 3 weeks. Thus, rapid turnaround may be needed. The mean age at onset of symptoms is 5 to 6 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 -70deg.C for 3 years; samples frozen at higher temperatures (-20deg.C) may lose activity, which may lead to inappropriate diagnosis of partial deficiency (B. Wolf, written communication, October 1994).

*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% to 95% accuracy.

*Accuracy of Screening Test*

False-Negative Rate. 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.

False-Positive Rate. 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.

Congential 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 to transform **cholesterol** to cortisol (hydrocortisone). These enzymes are: StAR / 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.

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 is caused by to the total absence of 21-hydrolase and can lead to the most severe salt losing form of the disease 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.

| **Salt-Losing CAH** | **Simple-Virilizing CAH** | **Late-Onset CAH** |
|---|---|---|
| a. No cortisol = hypoglycemia<br>b. No aldosterone = salt | a. Normal or near normal cortisol<br>b. Increased cortisol | a. Normal cortisol<br>b. Normal aldosterone<br>c. Increased 17- |

| | | |
|---|---|---|
| and water loss<br>c. Increased cortisol precursors (17-hydroxyprogesterone) = salt-losing tendency<br>d. Increased androgens = masculinization | precursors (17-hydroxyprogesterone)<br>c. Increased aldosterone to compensate for salt losing tendency<br>d. Increased androgens = masculinization | hydroxyprogesterone (moderate)<br>d. Increased androgens = masculinization |

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 of 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 is of 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 to 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.

**Sample collection / stability**: *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 turnaround 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 1 to 2 weeks of age detects some additional cases of simple virilizing CAH and increased numbers of the

nonclassic 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, available from many commercial laboratories. A relatively small sample of blood is required.

***Accuracy of Screening Test***

**False-Negative Rate**. Low: 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.

**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.

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 be harmful to 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 to receive Rh immunoglobulin post delivery, in order to prevent the development of maternal Rh-antibodies that could be harmful to 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 that are 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 negative test usually means and the infant is not at risk. A positive test means the baby is at risk of developing hemolytic anemia, and a negative test indicates that that the mother's antibodies are not reacting with the baby's blood and 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.

Limitation of direct antiglobulin test

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 while a positive result does not necessarily mean that the baby will develop anemia.

Chapter 10 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 bilological compounds involved the use of Gas chromatogaraphy 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 spec, these difficulties were overcome and the specialty analysis became available that was both fast and sensitive. It was initially used for specialized clinical testing to measure carnitine esters in the blood and urine of children suspected of inborn errors of metabolism.

Mass spectrometry separates and measures the mass to charge ratio of ions that have been produced from fragmentation of parent molecules in the ionization chamber of the mass spectrometer. The most common techniques consists of separation of the substances to be measured in a gas chromatograph, followed by fragmentation and measurement in a single mass spectrometer. .

The tandem mass spectrometer, abbreviated MS/MS 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, and is injected into the first quadrupole, which separates the *parent ions* from each other. The ions then 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 ionisation is a 'soft ionisation' technique which enables the direct analysis of biological high molecular weight substances like proteins previously considered non-candidates for mass spectrometry. Compounds can be detected and quantified directly from solution; there is no need to volatalise the sample. It offers excellent low sensitivity (femtomole detection limits). Because separation of compounds in the mixture is by mass

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spectrometry instead of 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 a-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 1 to 2 minutes, the system has adequate throughput to handle the large number of samples that are processed in newborn screening programs. Some conditions that can be diagnosed by MS/MS are listed in Table 1, together with the compound(s) on which diagnosis is based.

Table 1 Some disorders detectable by tandem mass spectrometry

| Disorder | Diagnostic metabolite |
|---|---|
| Amino acidemias | |
| Phenylketonuria | Phenylalanine & tyrosine |
| Maple syrup urine disease | Leucine 1 isoleucine |
| Homocystinuria (CBS deficiency) | Methionine |
| Citrullinemia | Citrulline |
| Hepatorenal tyrosinemia | Methionine & 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 |

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.

Chapter 11 Recommendations for the measurement of urine organic acids

Mike Bennett

Background:

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, presently untreatable conditions early diagnosis enables genetic counseling to be provided before multiple affected siblings are delivered.

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

Pre analytical 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 time of sample collection. 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. **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 minus 70°C and for several months at minus 20°C.

**We recommend that samples be stored at minus 20°C prior to analysis unless analysis is immediate when 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 results in between 0.5-3.0mL of urine to be extracted.

For extremes of concentration we recommend that the minimum volume to be extracted is 0.5mL and the maximum is 3.0mL.
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-phenylbuyric 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. 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 mini columns 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 data bases for organic acid spectra are based upon spectra generated from trimethylsilyl (TMS)-derivatives.

Gas Chromatography-mass spectrometry:

**We recommend that TMS derivatives of extracted urinary organic acids be prepared for GC-MS analysis.**

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

We recommend that an auto tune is performed daily and that analysis only proceeds if the tune falls within the specifications provided by the instrument manufacturer.

Choice of column: A variety of capillary GC columns are used for separation of organic acids with equivalent efficiency of separation. Columns are typically 25-30meters in length, 0.2-0.5mm 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.
We recommend that sample injection onto the column is 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. Typical and recommended GC temperatures are Injection port 240-250°C, Initial oven temperature 70-100°C, temperature ramp 3-8°C per minute, final oven temperature 270-295°C.
We recommend that the temperature of the mass spectrometer interface 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 between 30-60min.

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 0.5seconds. Depending upon the mass range of the mass spectrometer we recommend that the range of ions scanned 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. We recommend that 10-15 analytes be used in this calibrator mix and that they consist of significant compounds of diagnostic interest.

Data Interpretation:

Quantitative versus qualitative data analysis: Some laboratories provide extensive quantitative reports whilst 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.
**We recommend that for this purpose standard curves encompassing the reportable range for an analyte be generated at frequent intervals.**

For concentrations of organic acids less than 100mmol/mol creatinine:
**We recommend that quantitation is by isotope ratio mass spectrometry utilizing 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.
**We recommend that laboratories measuring urine organic acids participate in CAP activities and in addition, 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:

1. n-Hexanoylglycine, an important marker of medium-chain acyl CoA dehydrogenase deficiency.

Ethylmalonate, a marker for multiple disorders which frequently co-chromatographs with phosphate a quantitatively more significant compound.

Orotic acid, a marker for a number of urea cycle disorders, which frequently co-chromatographs with aconitate.

4-Hydroxybutyrate (gamma hydroxybutyrate) a marker for succinic semialdehyde dehydrogenase deficiency.

3-Hydroxyglutarate a marker for glutaric acidemia type 1.

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