Amniotic Fluid

- **Physiology, Composition and Formation**
  - Contained within the amnion
  - Formed by
    - Maternal circulation/plasma
    - Transfer of water across placental membrane
    - Metabolism of fetal cells
    - Fetal urine
  - Volume
    - Strasinger - @ 1L peak (@700-1200mL)

- **Amniotic Fluid**
  - Found in membranous sac surrounding fetus (Amnion)
  - Formed by
    - Maternal circulation / plasma (early)
    - Transfer of water across placental membrane
    - Metabolism of fetal cells
    - Fetal urine (later stages of development)
Amniotic Fluid

• Physiology, Composition and Formation
  • Volume 700-1200 mL term
  • Provides cushion to protect fetus, allows movement
  • Allows for exchange of water and chemicals to take place between fluid, fetus, and Mom

Amniotic Fluid

• Function – suspension and protection of fetus, medium for nutrients to be exchanged.

• Indications for analysis
  • Chromosomal abnormalities, such as Down's syndrome
  • Metabolic disorders, such as Tay Sachs
  • Neural tube defects – such as spinal bifida or an encephalic
  • Determination of extent of HDN
  • Others - gestational age, fetal maturity, etc.

Amniotic Fluid

• Specimen collection
  • Amniocentesis (using ultrasound)
  • 16-42 weeks gestation
  • @ 20-30 mL through fine needle collected in sterile syringes
  • Immediately transfer into sterile tubes (brown colored protection from light)
Amniotic Fluid

- **Color & Appearance**
  - Normally colorless
  - Some turbidity is normal - cellular debris, especially late in fetal development period.

- **Color & Appearance cont.**
  - Blood streaked - traumatic tap, abdominal hemorrhage, intra-amniotic hemorrhage
  - Yellow - bilirubin
  - Dark green - meconium
  - Dark red-brown - probable fetal death has occurred.
Amniotic Fluid

- Handling and processing
  - Special precautions
    - Specimens for bilirubin testing must be protected from light exposure and process immediately
    - Fetal lung maturity testing specimens must be kept cold until tested
    - Cytogenetic study specimens at RT or 37 degrees
    - Other chemistry tests require separation of cells, etc. from the fluid to preserve constituents.

- Laboratory procedures
  - HDN review:
    - Purpose of bilirubin testing on amniotic fluid:
      - Measurement of bilirubin is an indication of degree of hemolysis occurring in utero, therefore an indication of danger of anemia in the fetus.

- Bilirubin by spectrophotometric analysis
  - Scan fluid at increasing wavelengths
  - Plot readings against a baseline
  - Measure difference between baseline and peak bilirubin at 450 nm
  - Plot difference on Liley graph, against gestational age.
Amniotic Fluid

- Creatinine
  - Fetal age determination (at 36 weeks, fetal kidneys excrete >2.0 mg/dL creatinine)
  - This test has been replaced by ultrasound measurements.
  - Creatinine still used as:
    - Measurement as means of determining a fluid to be amniotic or urine.
    - Creatinine level up to 3.5 mg/dL & urea level @ 30 mg/dL can be found in amniotic fluid
    - Urine levels of creatinine @10 mg/dL and @ 300 mg/dL for urea.

Amniotic Fluid

- Alpha fetal protein (AFP)
  - Detection of neural tube defects (@1/500)
  - Perform test on amniotic fluid and Mom's blood
  - Follow up test (amniotic acetylcholinesterase) is more specific. Can not have blood contamination.
  - (AFP - also done on adults as one of many for detection of hepatocellular cancer or gonadal tumors.)

Amniotic Fluid

- Tests for Fetal Maturity - to determine whether fetus is capable of surviving an early delivery.
  - Major complication of early delivery - hyaline membrane disease (most common cause for death of premature newborn)
  - Lecithin / Sphingomyelin ratio
    - Ratio of 2:1 or more = lungs mature enough
    - Thin layer chromatography (TLC)
    - Blood, meconium, vaginal mucus interfere with test
  - Phosphatidyl glycerol
    - Latex agglutination or TLC
    - Fewer problems with contamination interference
Amniotic Fluid

- **Foam / shake test**
  - Bedside testing indicates total surfactant concentration
  - Series of tubes each containing increasing amount of 95% ethyl alcohol. Procedure on guide/text.
  - It evaluates the ability of pulmonary surfactant to generate a stable foam in the presence of ethanol.
  - Mix in amniotic fluid, shake and observe for ring of persistent bubbles around edge of meniscus
  - *Crude, but quick & cheap.*

Amniotic Fluid

- **Optical density** - evaluate the turbidity of fluid, based on thought that turbidity result of increased phospholipid concentration
  - OD greater than 0.150 indicates mature lungs (provided no contamination with blood or meconium).

Amniotic Fluid

- **Microviscosity**
  - Fluorescent dye binds with surfactants and albumin.
  - Test run on Abbot TDx and results correlate well with L / S ratio
Amniotic Fluid

- Lamellar bodies
  - "packets" of surfactant lipids produced by pneumocytes
  - Size 1-5 um (slightly smaller than platelets)
  - Can run on automated cell counters (platelet mode)

Amniotic Fluid

- Cytogenic analysis
  - Determination of chromosomal abnormalities and certain metabolic defects
  - Cells cultured
    - Chromosomes evaluated for appropriate number and completeness
    - Some cells lyed and contents analyzed for enzymes to evaluate for metabolic defects, such as Tay Sachs.
  - Not done on all patients
    - >35, or history of problems
    - Increased AFP
    - Known carriers.