



Topics in Pediatrics

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Inspiring Innovation and Discovery

Learning Objectives

- ▶ After this session the participant will be able to:
 - Recognize and manage issues related to pediatric testing (sample volume, interferences, interpretation)
 - Diagram methods for choosing or establishing pediatric reference intervals
 - Understand the usefulness of bilirubin and sweat tests in the neonate

Topics

- ▶ Sample volume, interferences
 - ▶ Reference intervals, critical values
 - ▶ Sweat test
 - ▶ Bilirubin
- 

Sample Volume

- ▶ **How much sample volume do you need?**
Minimum test volumes
- ▶ **Is this test necessary?**
 - ALT and AST ?
 - Hypoglycemia workup (ammonia, insulin, C-peptide, GH, cortisol, FFA, AA, acylcarnitine)

Pediatric volumes

How much sample volume can you draw?

How much volume does a baby have? < 1 pint!! (adults average 10pints (4.5 -5L))

What proportion of that can you safely draw?

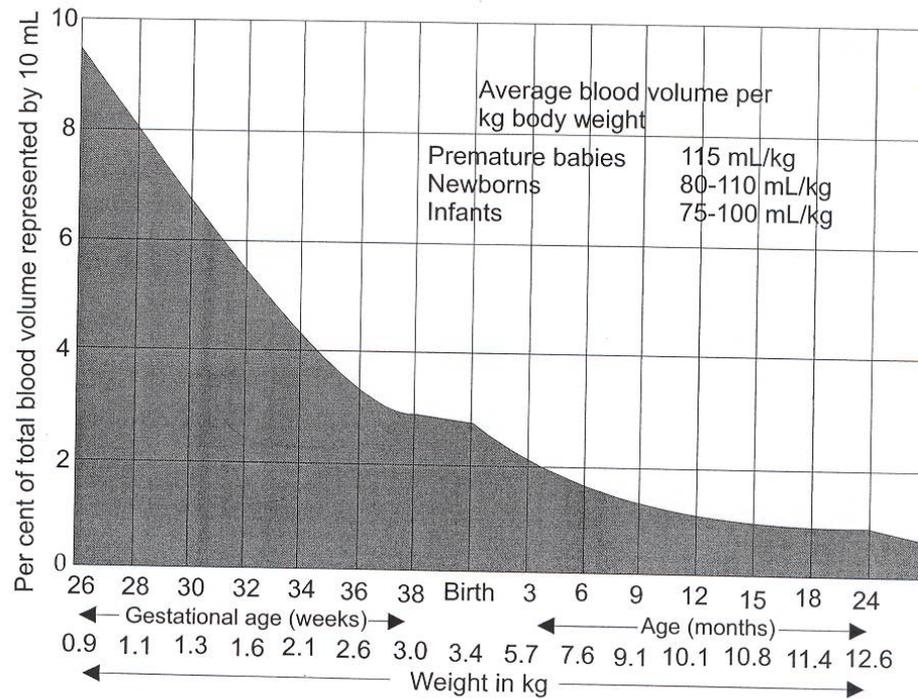


Figure 1.3 Relationship of a 10 mL blood sample to total blood volume



(Neonatology and Laboratory Medicine—Anne Green ,I.Morgan .J.Gray)

Hematocrit

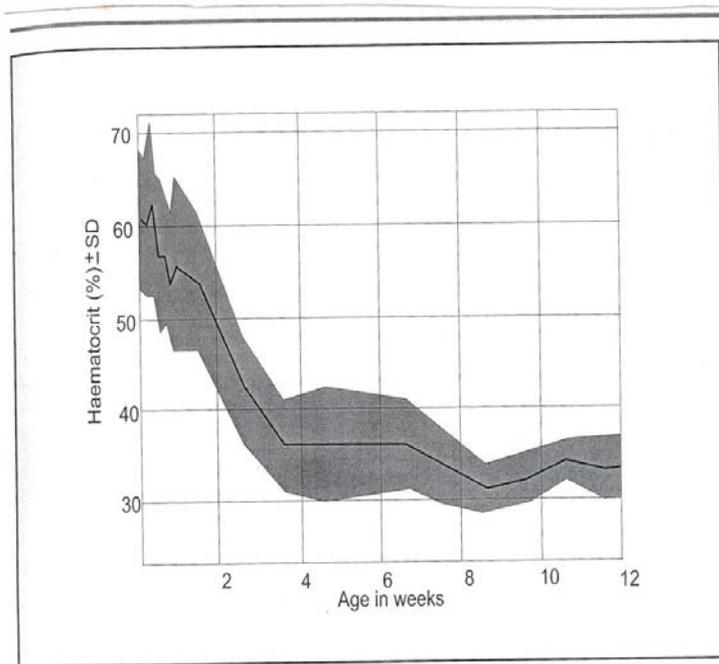


Figure 1.2 Haematocrit in the term neonate and young infant

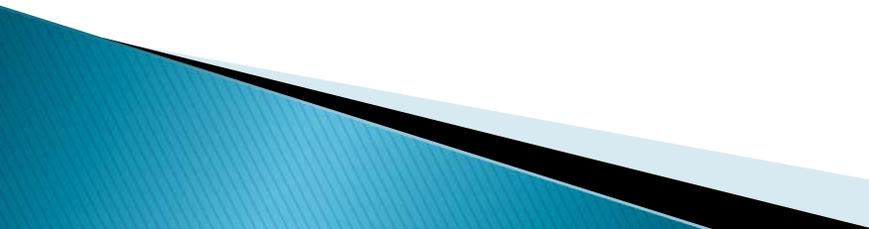


Small samples

▶ Heel stick samples

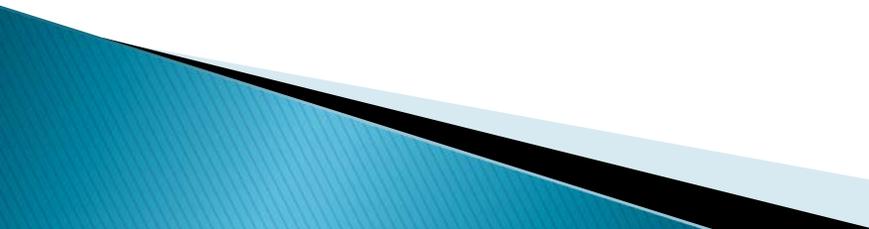
- Warm the heel to obtain free flowing capillary blood
- Use pedi-lancets $< 1.8\text{mm}$ long (1.5–3.2mm)
- Puncture the side of the heel. More distance between surface and bone (2 –8 mm)

Heel Stick or Veni-puncture

- ▶ For specimens >1 to 1.5cc
 - ▶ When skin contaminants could invalidate result e.g. blood cultures, trace elements
 - ▶ *Veni-puncture is preferred*
- 

Sample Volume

Small volumes and laboratory:

- ▶ **Policies– Mislabeled samples, Add-ons, hemolyzed samples**
 - ▶ **Instrumentation: Volume / test & Dead volume 0.6 –1.5 mL tubes**
 - ▶ **Primary tube sampling, Bar-coding, Robotics**
- 

Case

- ▶ A 2 year old girl is seen in the E.R. with suspected sepsis. Electrolytes were drawn:
 - Na 140 135–145 mEq/L (mmol/L)
 - K 6.9 *H 3.5–5.0 mEq/L (mmol/L)
 - Cl100 98–107 mEq/L (mmol/L)

Reference Intervals

- ▶ Age specific reference intervals
- ▶ Neonatal RI often differ from that of older children and adults

Tanner Stage RI

- ▶ **Steroid hormone concentrations related to developmental stage, not age**
 - ▶ **These values are helpful in endocrine testing where chronological age may not reflect developmental stage**
- 

Phosphate

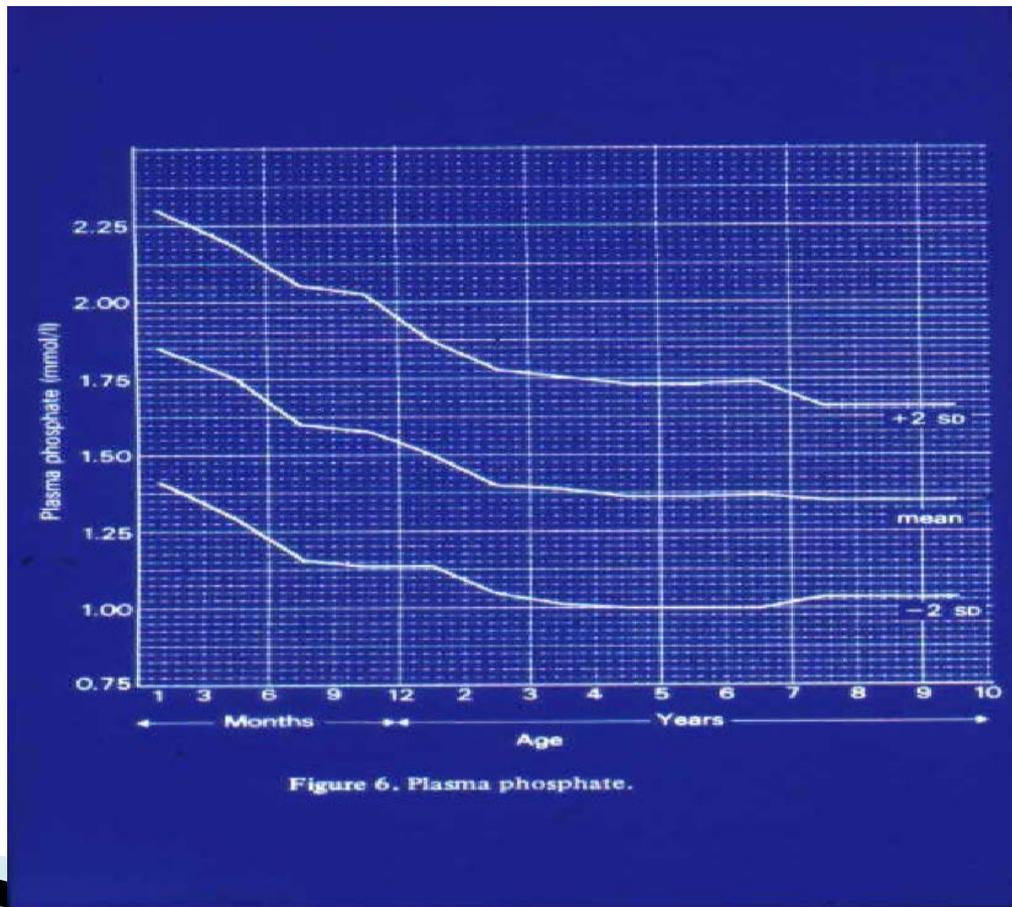
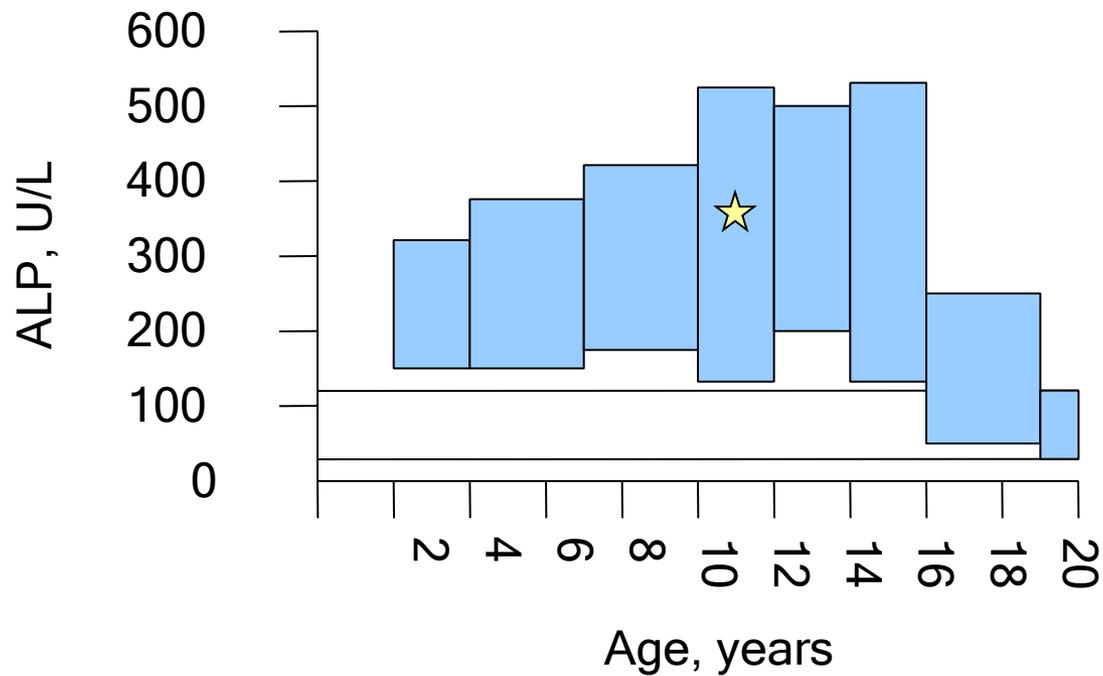


Figure 6. Plasma phosphate.

Case

- ▶ A 13 year old boy wishes to start Acutane for acne. He has previously been healthy. The family physician orders some blood work prior to starting the medication:
 - AST 21 <35 U/L
 - ALT 18 <35 U/L
 - ALP 320 H 30-120 U/L
 - GGT 30 <45 U/L

Reference Intervals



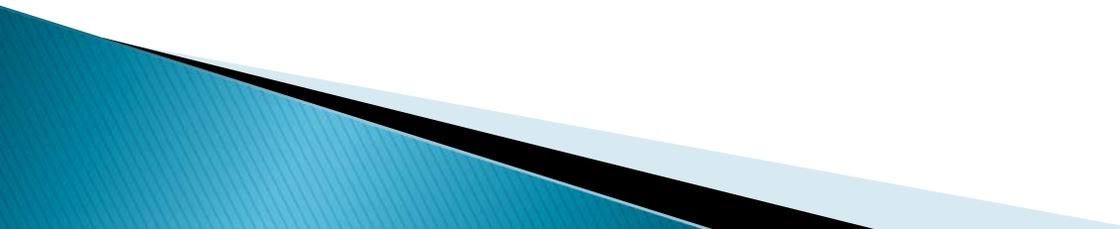
Lockitch G *et al.* 1988 *Clin. Chem.* 34(8): 1622

Establishing reference intervals

Very specific rules for establishing RI

- ▶ IFCC Expert Panel on Theory of Reference Values. Approved Recommendation on the Theory of Reference Values. (6 parts published in J Clin Chem Clin Biochem, 1987--1991)
- ▶ How to Define and Determine Reference Intervals in the Clinical Laboratory: Approved Guideline Second ed. CLSI C28--A2

Three basic rules:

- ▶ Samples from reference individuals. (healthy)
 - ▶ $N = 120$ for each subgroup
 - ▶ Use non-parametric analysis
- 

Establishing pediatric intervals

Inherent hurdles

- ▶ Ideally 120 samples for each age group; Minimum of 40 for 95% intervals
 - ▬
- ▶ Healthy individuals
- ▶ Consent of parent/guardian;
 - Strict IRB constraints
- ▶ Enough sample
 - Infants and young ages: Always the most difficult

PRI studies on-going (North America)

- ▶ **CALIPER–**
Canadian Laboratory Initiative on Pediatric Reference Intervals (*April 2013* (Clin Biochem), May 2012, (Clin Chem), June 2011 (Clin Biochem))
- ▶ **ARUP –** extensive intervals being done (Caucasian, local to Utah)
- ▶ **AACC– PRRC –** collaboration with NCS (DBS) (steroid hormones, amino acids)

Methods of establishing intervals

- ▶ **Methods for using small numbers of samples** Horn PS, Pesce AJ. Reference Intervals: a Users Guide. AACCC Press, 2005
- ▶ **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory : Approved Guideline Third ed. CLSI C28-A3**
- ▶ **ROBUST method**
- ▶ **“Validate” and “transfer” RI rather than validate**

Validating intervals

- ▶ **Transference still requires 20 samples /age group**
- ▶ **The Hoffman approach**
 - **Better for validating rather establish**
 - **Uses hospital based data**

Critical Values

- ▶ These are different in children
- ▶ Should be established in consultation with your clinical colleagues.

Bilirubin

- ▶ Neonatal Hyperbilirubinemia

Neonatal hyperbilirubinemia

- ▶ Unconjugated – ‘neonatal jaundice’
 - **Immature liver enzymes**
 - Shortened RBC life and increased production
 - Breast-feeding – α -glucuronidase in breast milk unconjugates bili and allows it to be re-absorbed (affects about 30% of breast-fed infants)

Neonatal jaundice

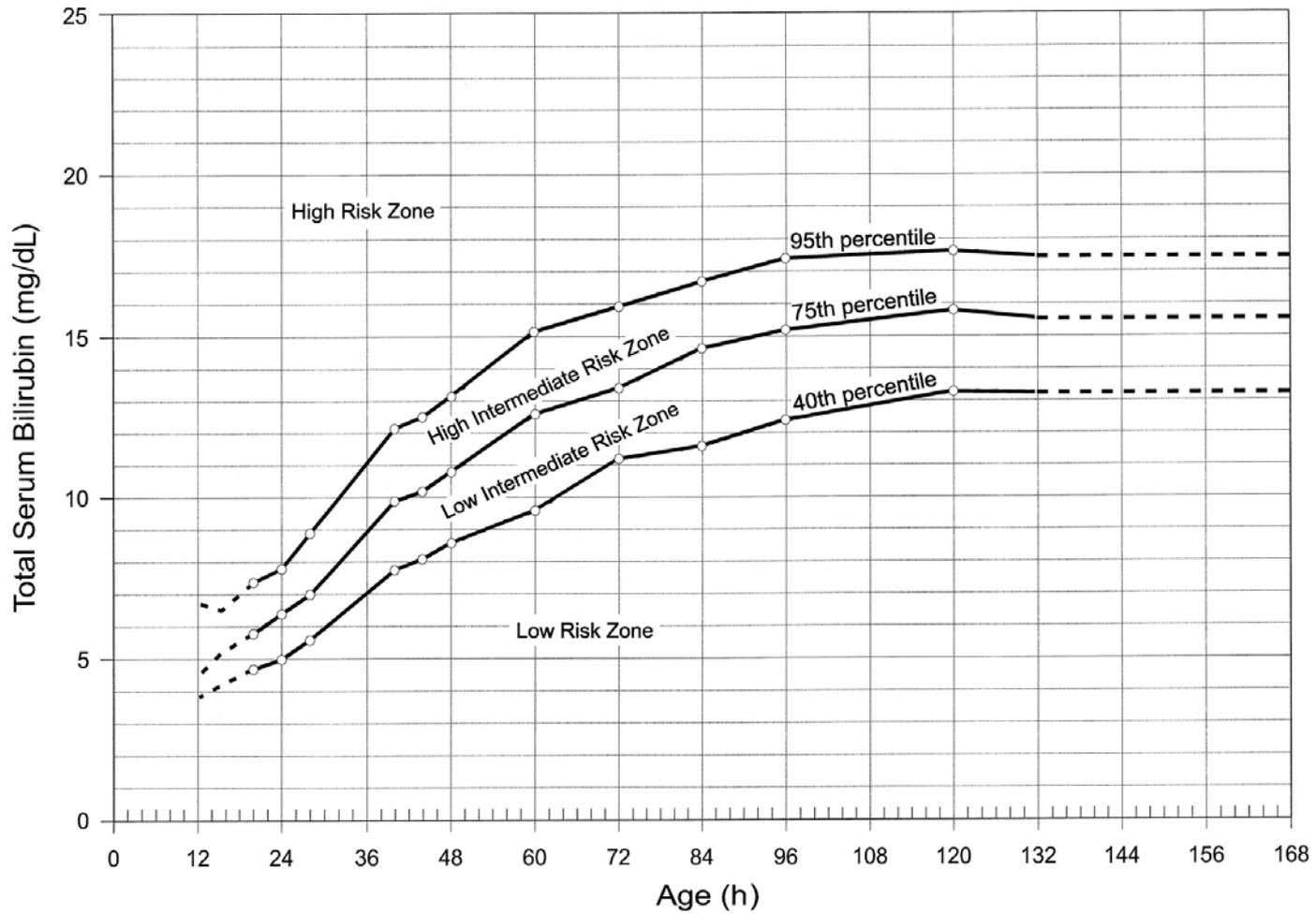
- ▶ Jaundice is common in the newborn
- ▶ *Peak TSB levels around the 4th–5th day of life ; discharge is 48h .*
- ▶ Prolonged increase
 - Hemolytic disorders, G6PD deficiency, birth trauma, breast feeding, prematurity
- ▶ 2% will have $>20\text{mg/dL}$ ($340\ \mu\text{mol/L}$)

Kernicterus

- ▶ Critical hyperbilirubinemia >25 mg/dL (425 $\mu\text{mol/L}$)
- ▶ Increased risk of kernicterus
 - Detrimental neurological defects
 - auditory dysfunction, intellectual and other handicaps

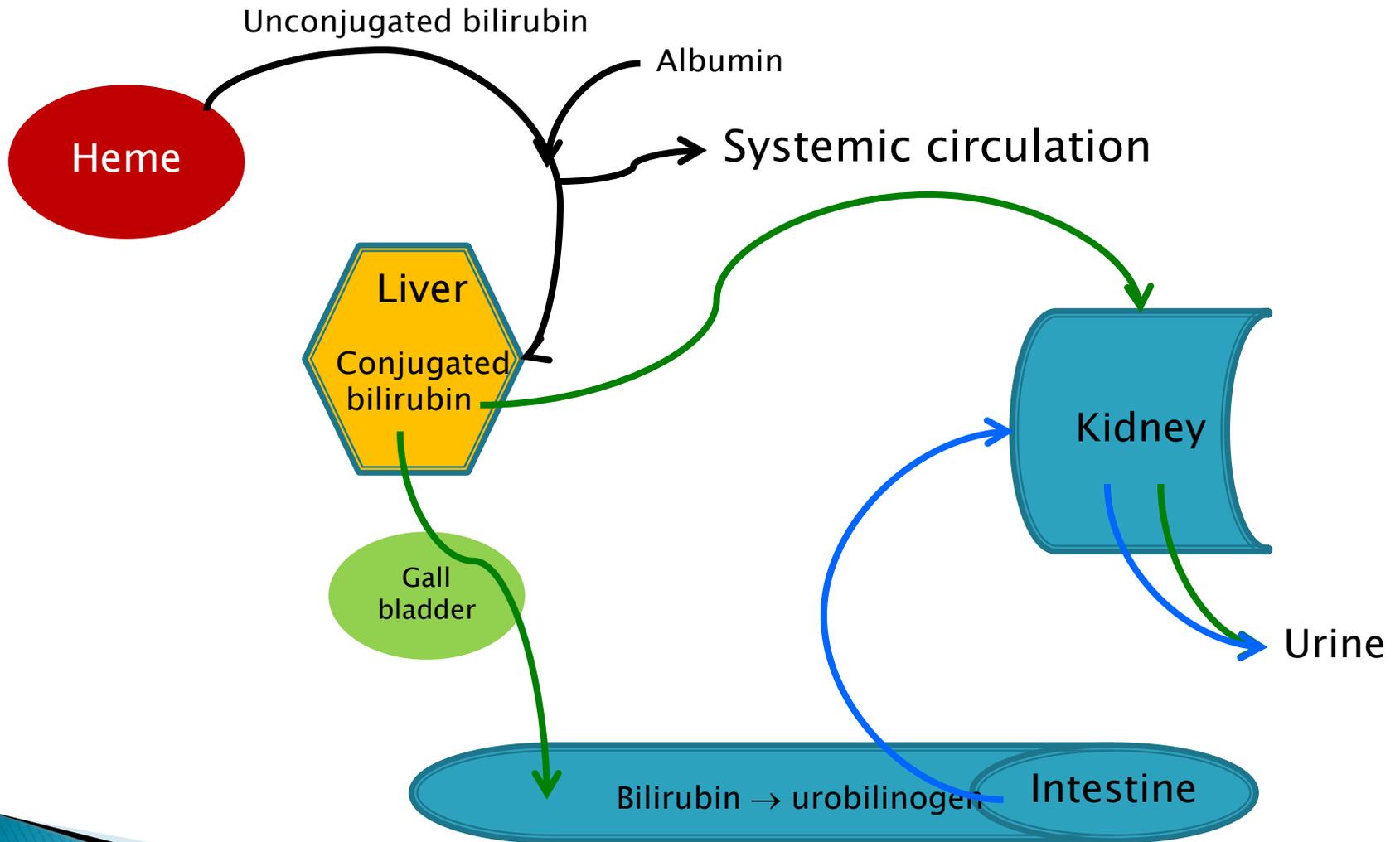
2004–AAP management guidelines

Assess for jaundice prior to discharge and provide appropriate follow-up

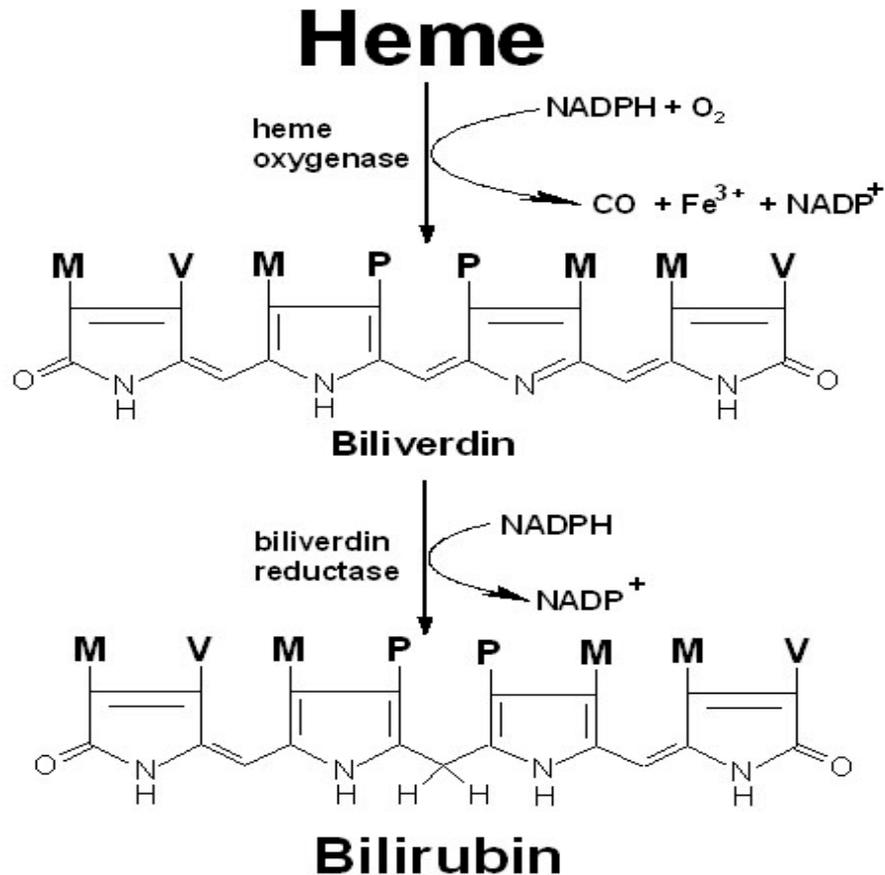


Bhutani, V. K. et al. Pediatrics 1999;103:6-14

Bilirubin Metabolism

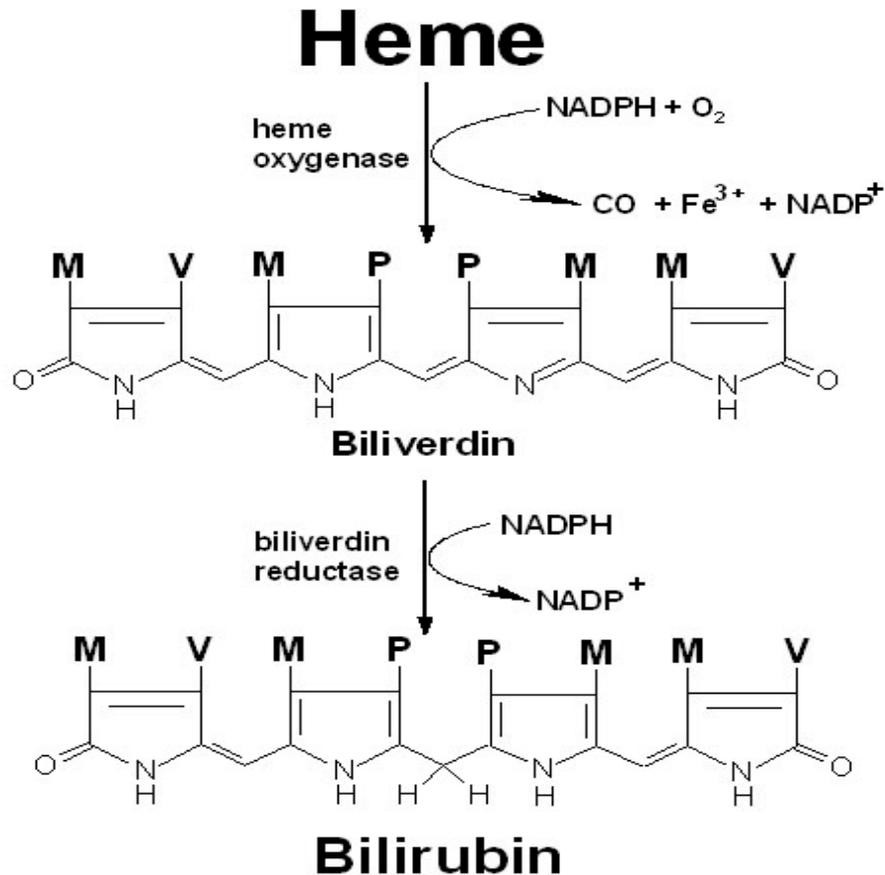


Bilirubin Metabolism



M = methyl = -CH₃
V = ethene = -CH=CH₂
P = propionic acid =
CH₃CH₂COOH

Bilirubin Metabolism



M = methyl = $-\text{CH}_3$
V = ethene = $-\text{CH}=\text{CH}_2$
P = propionic acid = $\text{CH}_3\text{CH}_2\text{COOH}$

Bilirubin

- ▶ **Direct Bilirubin**
- ▶ **water soluble { mono-and di-conjugates, protein bound}**
- ▶ **Total Bilirubin –**
 - All bilirubin fractions
- ▶ **Total –**
Direct = Unconjugated bilirubin (Not directly measured by diazo methods)

Bilirubin measurement history

- ▶ 1883 – Ehrlich introduced the diazo reaction for the detection of bilirubin
 - ▶ 1916 – Van den Bergh and Muller described and coined the terms “direct” reacting and “indirect” being total minus direct bilirubin
 - ▶ 1937 – Malloy and Evelyn adapted the bilirubin procedure to the photoelectric colorimeter
 - ▶ 1938 – Jendrassik and Grof described the basis of our current diazo methods – reproducible and reliable
- 

Measurement

- ▶ **Jendrassik and Grof –Diazo**
 - **Diazo salts react to give Direct Bilirubin**
- ▶ **Add an accelerant to the sample**
(Early on was ethanol, then caffeine or benzoate)
React with diazo–
salts again to give Total Bilirubin

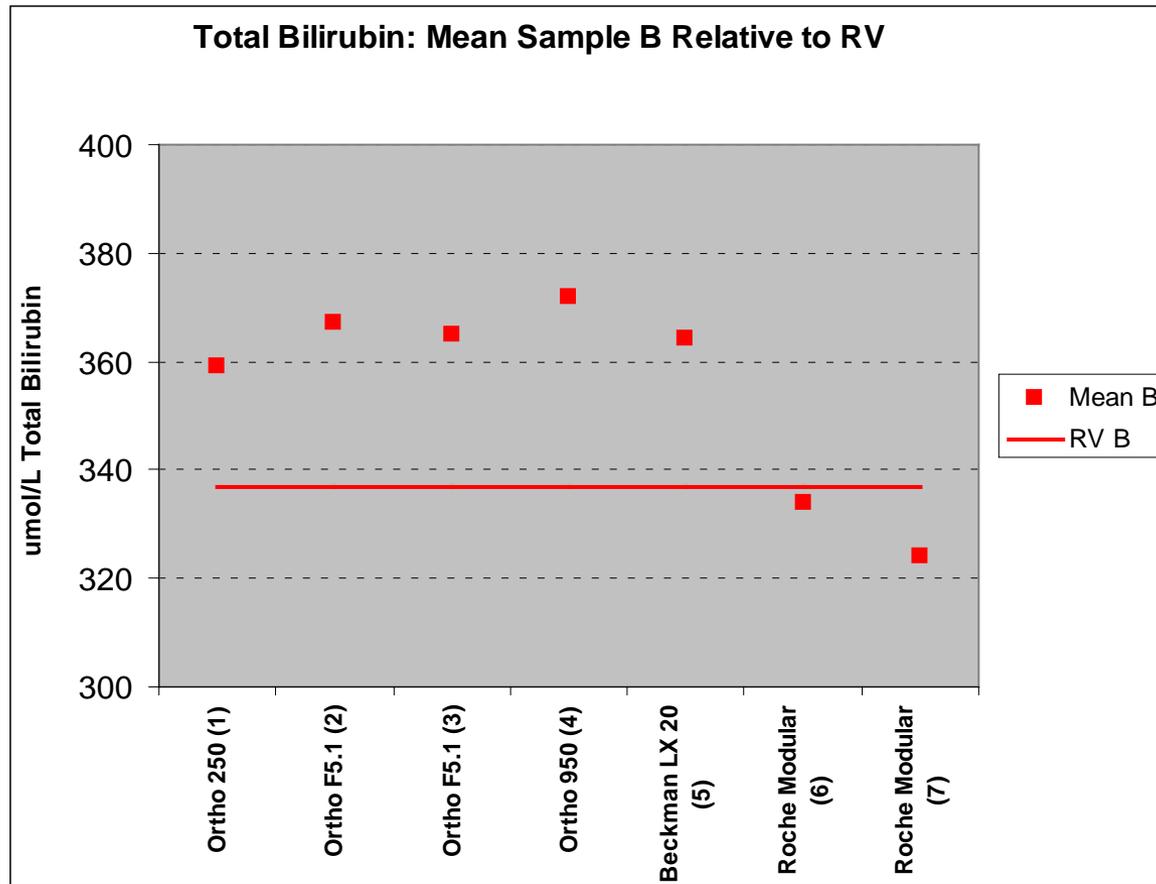
Measurement

- ▶ **Ortho Diagnostics Vitros dry-slide assay**
 - Measures Bc and Bu by reflectance spectrophotometry
 - directly measures the unconjugated and conjugated
- ▶ Uses a mordant to shift maximum absorbance peak Wu, et al. Clin Chem 30(8):1304-9, 1984

TSB or TcB

- ▶ Bhutani Nomogram is TSB
 - ▶ Diazo method (Hitachi 747)
 - ▶ How do laboratory methods compare?
- 

TSB values from 7 labs (Canada)

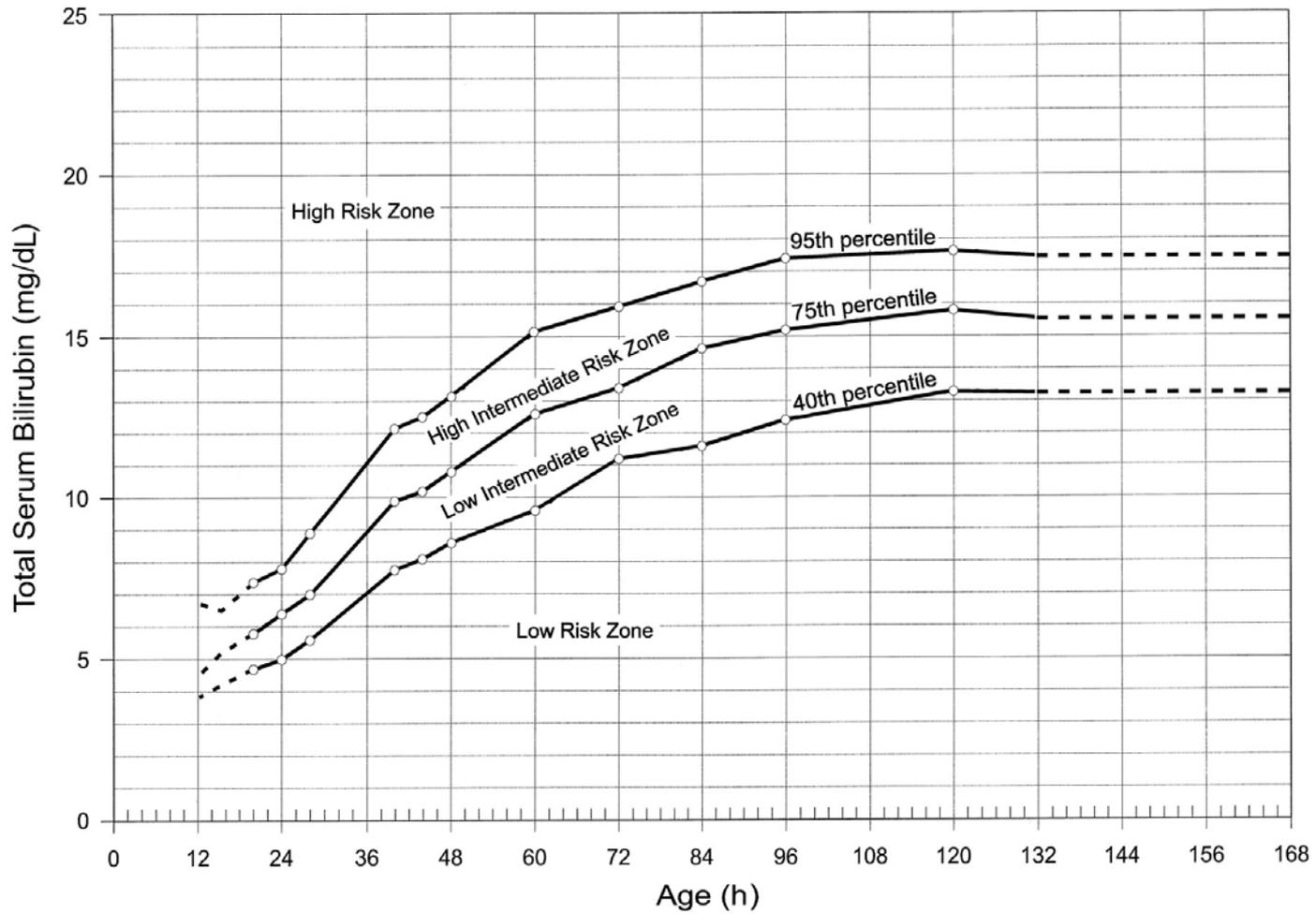


Case

- ▶ Baby boy F, 39 3/7 wks gestation, 3350g, Caucasian, mother is O⁺, Coombs negative.

At 24h TSB = 10mg/dL (174 μ mol/L (high risk))

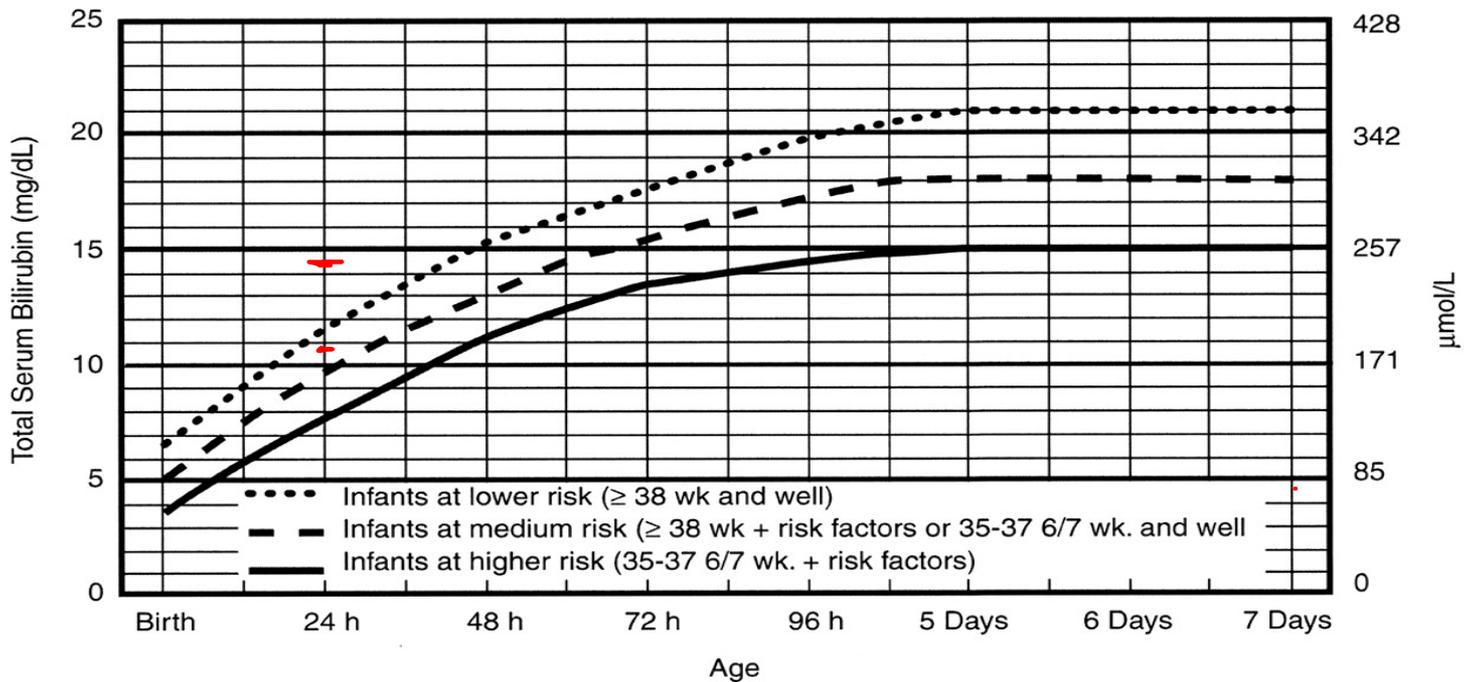
– Requires f/u but below low risk for initiation of phototherapy 11mg/dL (190 μ mol/L)



Bhutani, V. K. et al. Pediatrics 1999;103:6-14

Phototherapy Nomogram

(www.bilitool.org)



- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin.
- Risk factors = isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis, or albumin $< 3.0\text{g/dL}$ (if measured)
- For well infants 35-37 6/7 wk can adjust TSB levels for intervention around the medium risk line. It is an option to intervene at lower TSB levels for infants closer to 35 wks and at higher TSB levels for those closer to 37 6/7 wk.
- It is an option to provide conventional phototherapy in hospital or at home at TSB levels 2-3 mg/dL (35-50mmol/L) below those shown but home phototherapy should not be used in any infant with risk factors.

Case

- ▶ Next morning bilirubin is 254 $\mu\text{mol/L}$, now in high risk on screening nomogram, and above low risk for initiation of phototherapy
 - Phototherapy initiated and baby's course routine
 - Parents were upset that phototherapy not initiated sooner.

Low Risk

- ▶ 40th percentile is conservative
 - Variability in TSB measurements
 - 35/36 wks gestation may be discharged as if they are term babies.

Causes of severe hyperbilirubinemia

- ▶ ABO incompatibility
 - ▶ G6PD deficiency
 - ▶ Other antibody incompatibility
 - ▶ Hereditary spherocytosis
 - ▶ UTI
 - ▶ sepsis
- 

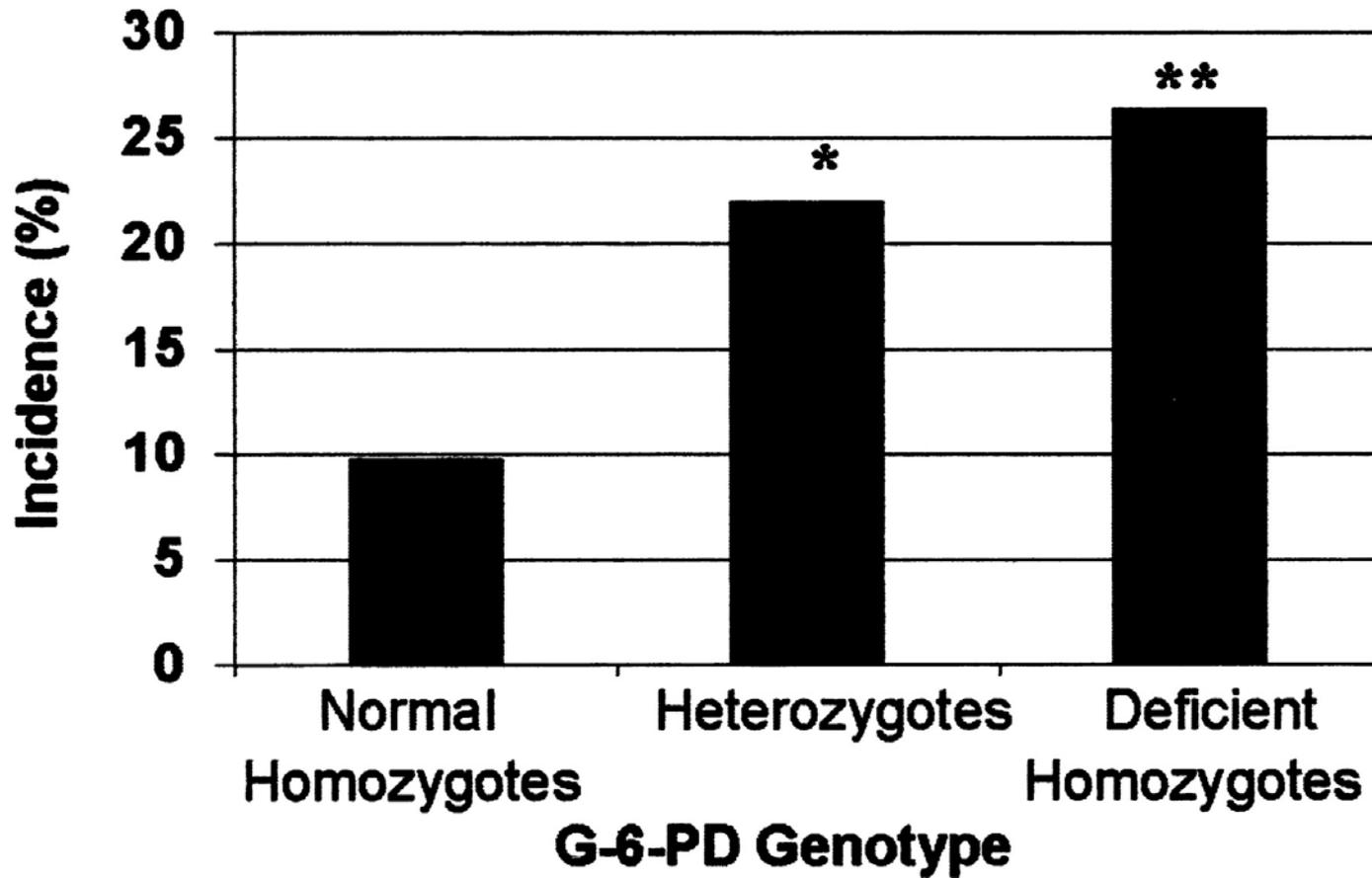
COOMBS test

- ▶ OR is 2.9 for severe hyperbilirubinemia when mother is blood group O and baby is blood group A or B.
 - ▶ Need for phototherapy increased
 - ▶ Blood group and a DAT done on all infants with early jaundice and mother with group O
- 

G-6PD

- ▶ 20/93 cases of severe hyperbilirubinemia
- ▶ Screen at-risk infants
 - African American, South-east Asian, Greek, Italian, Sephardic Jews, Chinese

Incidence of hyperbilirubinemia



Kaplan, M. et al. Pediatrics 1999;104:68-74

Disorders of bilirubin metabolism

- ▶ Two basic types of hyperbilirubinemia
 - Unconjugated Hyperbilirubinemia (UHB)
 - Three main causes
 - *Conjugated Hyperbilirubinemia (CHB)*
 - *Two main causes*

Always assess for CHB also

Sweat Test

- ▶ Why do we still need this test?

Diagnosis of CF

- ▶ One or more phenotypic features
- ▶ History of CF in sibling
- ▶ Positive newborn screen

And

- Evidence of CFTR abnormality– elevated sweat chloride concentration, or identification of two mutations known to cause CF.

Facts about CF

- ▶ Common recessive genetic disorder
 - ▶ 1:3200 Caucasians, – 1–31,000 in Asian Americans
 - ▶ CFTR mutations lead to clinical manifestations seen in CF
 - ▶ More than 1800 mutations have been identified
- 

Genotype and Phenotype

The $\Delta F508$ mutation appears to be associated with the “classic” form of CF:

- 1) high level of Cl^- in sweat
- 2) pancreatic insufficiency

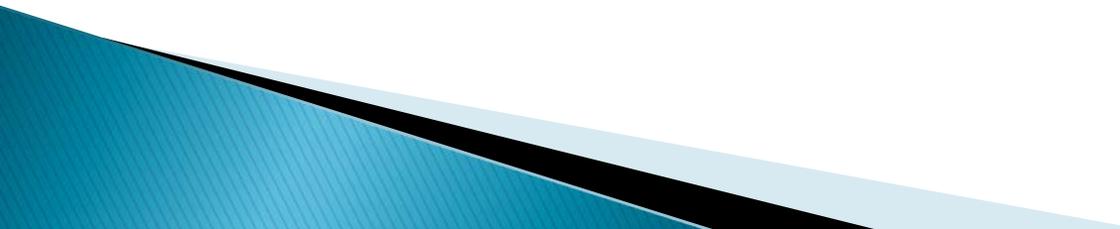
However, pulmonary disease -- the life-limiting feature of CF -- appears to be variable in those with this common mutation.

This reflects the **phenotypic variation** of the $\Delta F508$ mutation.

Sweat test

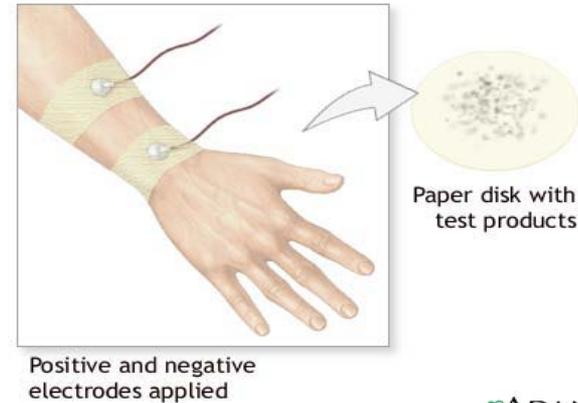
- ▶ QPIT remains the gold standard for diagnosis
- ▶ Guidelines for sweat testing
 - CLSI
 - CAP
 - –checklist: www.cap.org
 - USCF
 - UK
 - Australia

Key points

- ▶ Subject suitability
 - ▶ Sweat collection
 - ▶ Sweat analysis
 - ▶ Result reporting
 - ▶ Quality
- 

Collection

- ▶ CLSI guidelines
- ▶ QPIT
- ▶ Use only arms and legs
- ▶ Minimum 15 μL (Macroduct coil)
 - weight 75mg(Gibson–Cooke)



Collection

- ▶ Collect for no more than 30minutes
- ▶ Evaporation or contamination of sample
- ▶ Collect in duplicate (optional)
 - (agreement is 10mmol/L <60; 15 >60)
- ▶ Do not pool samples for analysis
 - (analysis of insufficient collections may result in FP or FN)

Analysis

- ▶ Chloridometer– coulometric titration
 - ▶ Manual titration
 - ▶ Automated analyzer–validated method

 - ▶ 2 levels of controls should be used–(CV 5%).
 - ▶ Labs should participate in EQA–(CAP)
- 

Analysis

- ▶ Analytical methods requiring the addition of extraneous chloride standard to patient samples should not be used.
- ▶ The limit of detection should be ≤ 10 mEq/L (mmol/L).
- ▶ Values > 160 mEq/L (mmol/L) should not be reported—recheck

Reference intervals

- ▶ Negative < 40 mEq/L (mmol/L)
- ▶ Borderline > 40 and < 60 mEq/L (mmol/L)
- ▶ Positive > 60 mEq/L (mmol/L)
- ▶ Neonates < 30¹ mEq/L (mmol/L)
considered abnormal in neonates and may require further evaluation

• ¹Farrell, Koscik, Pediatrics, 1996;97:524–528

Suitability

▶ US-CF

- Minimum age >48h or wt >2.kg
- Sweat chloride transiently elevated at age 24h +

▶ UK guidelines

- 2weeks or >3kg

<2.0kg

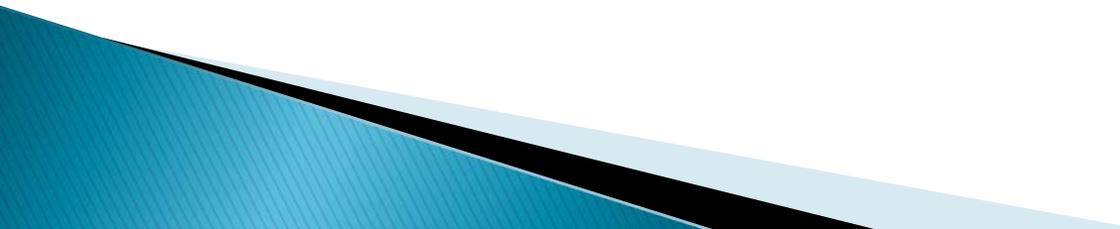
<38 wks gestation

Or A-A race

increased likelihood of NSQ

Delay testing in subjects on systemic steroids, edematous, dehydrated.

Repeat testing

- ▶ Positive tests should be confirmed with a repeat sweat test or another diagnostic test
 - ▶ CFTR mutation analysis can be the repeat test, but many mutations may not be picked up by the limited analysis
 - ▶ Borderline tests should be repeated.
- 

Quality

- ▶ Document % QNS–
 - failed tests should not exceed 10%, ideal <5%
- ▶ Document borderline and positive results
- ▶ Adverse skin reactions.

Personnel

- ▶ Sweat test should be performed by a limited number of experienced well-trained personnel who pass documented competency testing
 - ▶ UK guidelines suggest a laboratory should perform 50/annum, and personnel 10 tests/year.
- 

Summary

- ▶ Small sample volumes influence lab testing in pediatrics
 - ▶ Age or developmental-related reference intervals are necessary but are not easily available
 - ▶ Sweat Chloride is important for the diagnosis of CF
 - ▶ Bilirubin assessment has important implications in the neonatal period
- 

Self Assessment

10mL of blood is approximately

- ▶ 5%
- ▶ 10%
- ▶ 0.2%
- ▶ **2.5%**

of blood volume in a term baby weighing 4kg



Self Assessment

- ▶ CFTR mutation analysis can replace sweat testing in the diagnosis of cystic fibrosis
 - True or False

Self Assessment

- ▶ Hyperbilirubinemia in the neonate is
 - Assumed to be unconjugated bilirubin
 - Should be assessed for both conjugated and unconjugated bilirubin
 - Is caused by G6PD deficiency
 - **All of the above**