Transcutaneous Bilirubin: How Does it Compare to the Lab?

SW Florida Point of Care Network
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DISCLOSURE

Relevant Financial Relationship(s)

None

Off Label Usage

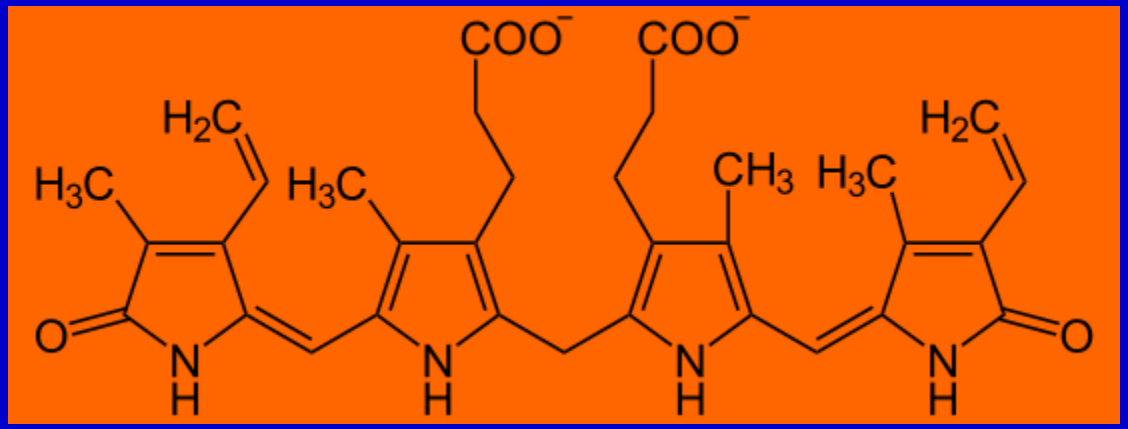
None

Outline

- Bilirubin
  - Production
  - Accumulation
- Bilirubin measurement
  - Reference method
  - Diazo methods
  - Peroxidase (unbound bilirubin)
  - Whole blood methods
  - Bu and Bc
- Intra and Inter-lab method comparability
- Relationship between TcB and lab bilirubin

Bilirubin

- Principle breakdown product of heme
- Produced primarily in liver, spleen, bone marrow
- Highly insoluble



Bilirubin

- **Accumulation**

  Adults: conjugated bilirubin >> unconjugated

  Infants: Unconjugated bilirubin peaks between 3-6 days of life

  - **RBC fragility**
  - **Immaturity of glucoronosyl transferase enzymes**
  - **Other factors**

- **High unbound bilirubin levels toxic**

  Kernicterus—frequency unknown, 100% preventable

Current guidelines

- AAP 2004: Assess every newborn for the risk of HB before D/C
  - 2 options:
    - Pre-discharge TSB or TcB- plot on age-based nomogram
    - Assess clinical risk factors
- US Preventive Services Task Force 2009
  - No evidence screening reduces Kernicterus
  - Possible harm thru overtreatment, increase cost

Bilirubin measurement

- Chromatographic separation identified 4 fractions:

  Unconjugated bilirubin

  Bilirubin monoglucuronide (singly conjugated)

  Bilirubin diglucuronide (doubly conjugated)

  Delta bilirubin (covalently bound to albumin)

Bilirubin measurement

- **Reference method**

  Doumas bilirubin method based on Jendrassik-Grof reaction

  Reaction of bilirubin with diazotized sulfanilic acid (diazo reagent) to form azobilirubin dye

  Accelerated by caffeine and sodium benzoate

  USA reference lab Medical College Wisconsin

  Reaction produces consistent results between labs
  Inter-lab CV ≤ 2% for values over 2 mg/dL
  Lo et al., Clin Biochem 2009

Bilirubin measurement

- **Diazo methods**

  Most common method used in labs

  Without accelerant *mainly* conjugated and delta bilirubin react with diazo reagent—direct bilirubin

  With accelerant all bilirubin reacts with diazo reagent—total bilirubin

    Total minus direct= indirect bilirubin
    Approximates unconjugated bilirubin

Bilirubin measurement

- **Peroxidase (unbound bilirubin) methods**

  Horseradish peroxidase catalyzes oxidation of unconjugated bilirubin

  Protein-bound bilirubin is protected

  Used as measure of unbound (free) bilirubin

  Hemolysis, conjugated bilirubin, photoisomers, sample dilution, peroxidase concentration may all interfere/impact performance of assays

Bilirubin measurement

- **Whole blood methods--photometric**
  - **Bilirubin max absorbance ~ 450 nm**
  - **Neonatal blood devoid of other substances that absorb near 450 nm**
    - **Makes direct measurement easier in neonates**
  - **Hemoglobin abs. similar at 450 and 540 nm**
    - **Abs. 450 minus abs. 540 = bilirubin**
  - **Both two-point (requires centrifugation) and multi-point wavelength analyzers exist**
    - **Multi-point whole blood bilirubin measurement on many blood gas/cooximetry analyzers**

Bilirubin measurement

- **Bu/Bc measurement (neonatal bilirubin)**

  Vitros dry slide technology

  Mordant separates absorbance spectra of conjugated and unconjugated bilirubin

  Upper layers of slide filter out hemoglobin, protein (delta bilirubin), other interferences

  Conjugated and unconjugated bilirubin measured separately

  Total neonatal bilirubin = conj + unconj

Bilirubin measurement

- **Bu/Bc measurement**

  Direct photometric measurement of both unconjugated and total (minus delta) bilirubin

  Less sensitive to effects of hemolysis

    Newer diazo methods also less sensitive
    Direct bili by diazo still problematic

  Small sample volume

Intra-lab comparability of methods

- Comparison of 9 methods within one academic medical center

  Grohman et al., Pediatrics 2006;117:1174-83.

  3 central lab methods

  Hitachi 912, Dimension RxL, Vitros 250

  3 whole blood methods

  Twin Beam, OMNI S, ABL 735

  3 transcutaneous methods

  JM-102, JM-103, BiliCheck

Intra-lab comparability of methods

- Comparison of 9 methods within one academic medical center

  124 samples from 122 near-term/term infants

  Plasma bilirubin conc. 0.5-22.7 mg/dL

    9 infants (7%) above 15 mg/dL

  3 lab methods correlated strongly

    Slopes 0.94-1.05 between all lab methods

    Intercepts < 0.2 mg/dL between all methods

Intra-lab comparability of methods

- Comparison of 9 methods within one academic medical center
  - Compared to mean of 3 lab methods:
  - 3 transcutaneous devices correlated well
    - Slopes 0.95-1.04
    - Intercepts -0.4 – 0.9 mg/dL
    - Correlation coefficients 0.961 to 0.966
  - 3 whole blood devices correlated well
    - Slopes 0.97-1.03
    - Intercepts -1.0 to 0.1 mg/dL
    - Correlation coefficients 0.980 to 0.994

Intra-lab comparability of methods

- **Comparison of 9 methods within one academic medical center**

  General conclusions (comparability)

  Laboratory, whole blood and transcutaneous bilirubin methods agree well up to ~ 12-15 mg/dL

  Can screen with either transcutaneous or whole blood bilirubin

  Confirm in lab above 12 (TcB) or 15 (whole blood) mg/dL

  For lower values doesn't matter which method used

Inter-lab comparability of methods

- **Lo et al., Arch Pathol Lab Med 2008;132:1781-5**

  Reported distribution of values from ~ 5000 labs participating in bilirubin Proficiency Testing

  10 lab methods including diazo, oxidase, photometric

  One sample/survey prepared by adding NIST SRM 916 to pooled human sera

    Reference Doumas method used to grade all other methods

Inter-lab comparability of methods

- **Lo et al.**

  Between 2003-2006 PT surveys contained one sample with reference value 19-22 mg/dL

  In 2003 mean all method bias ~ 0.5 mg/dL

  Mean bias (all methods) 1.5-2.0 mg/dL between 2003-2005, ~ 1.0 mg/dL in 2006

  In 2006 most major methods overestimated total bilirubin by ~ 1.0 mg/dL

  A few instruments differed widely from all others

Inter-lab comparability of methods

- Lo et al.
- Instrument and method-dependent differences in calibrators responsible for positive bias

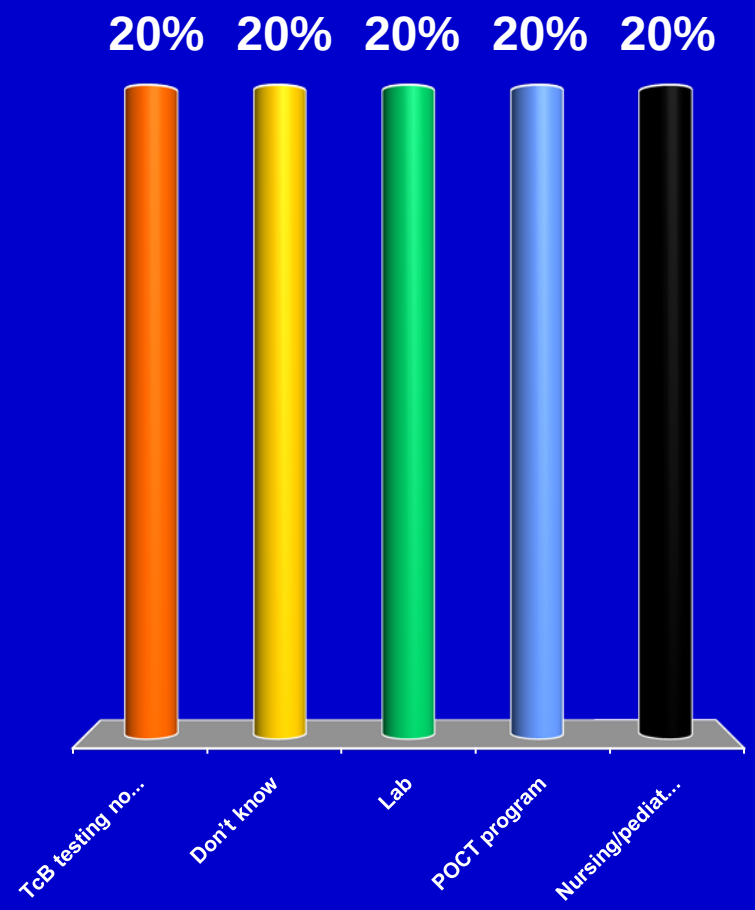
  Assigned calibrator values too high
- Proposed goal of 10% total error for bilirubin values > 10 mg/dL
- To reach goal quality of calibrators will need to be improved

Transcutaneous bilirubin

- Can TcB be used to screen for or assess risk of severe hyperbilirubinemia?
- What is relationship between TcB and lab bilirubin?
- Which lab bilirubin, does it matter?

At your institution who has oversight of TcB testing?

1. TcB testing not done
2. Nursing/pediatric service
3. Lab
4. POCT program
5. Don’t know



TcB

- 4 studies BiliChek TcB underestimates serum bilirubin by 0.06-0.96 mg/dL
- 1 study BiliChek TcB overestimates serum bilirubin by ~ 1 mg/dL across a wide range of serum bilirubin values
- 2 studies BiliChek TcB overestimates serum bilirubin at low concentrations, underestimates serum bilirubin at higher (> 12 mg/dL) levels
- No consistent association between method (photometric, diazo, BuBc) and mean bias

TcB

- **Questions about TcB**

  What is sensitivity and specificity of high risk TcB for predicting high risk TsB?

  If TcB is low risk, can we avoid blood draw (high sensitivity)?

TcB

- **Mayo study**

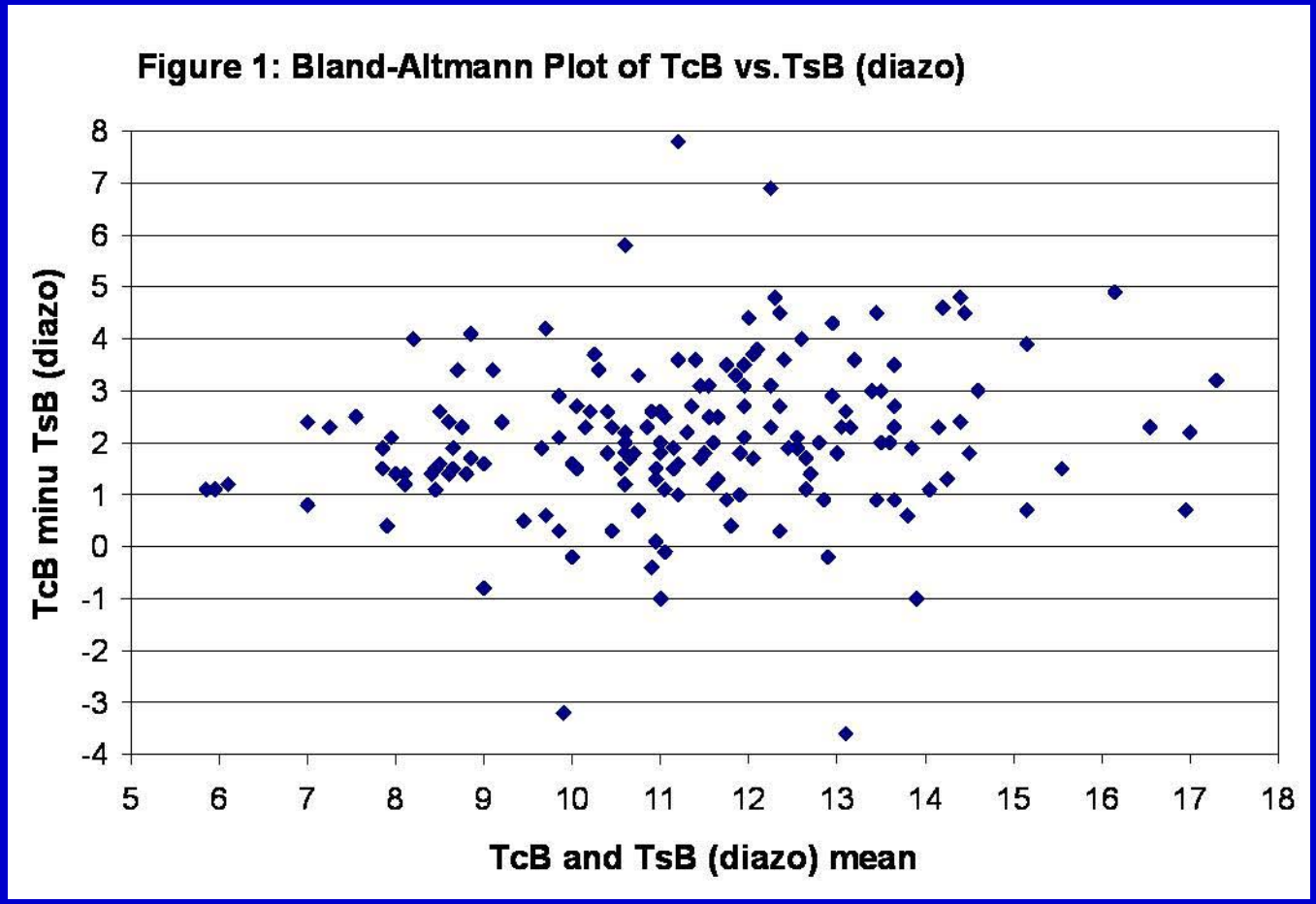
  200 infants with clinical suspicion of hyperbilirubinemia, serum bilirubin ordered by physician in nursery

  Measure BiliChek TcB within 30 minutes of serum bilirubin drawn

  Measure serum bilirubin diazo method and direct photometric measurement of unconjugated bilirubin (BuBc)

TcB

Results: TcB vs. diazo TsB

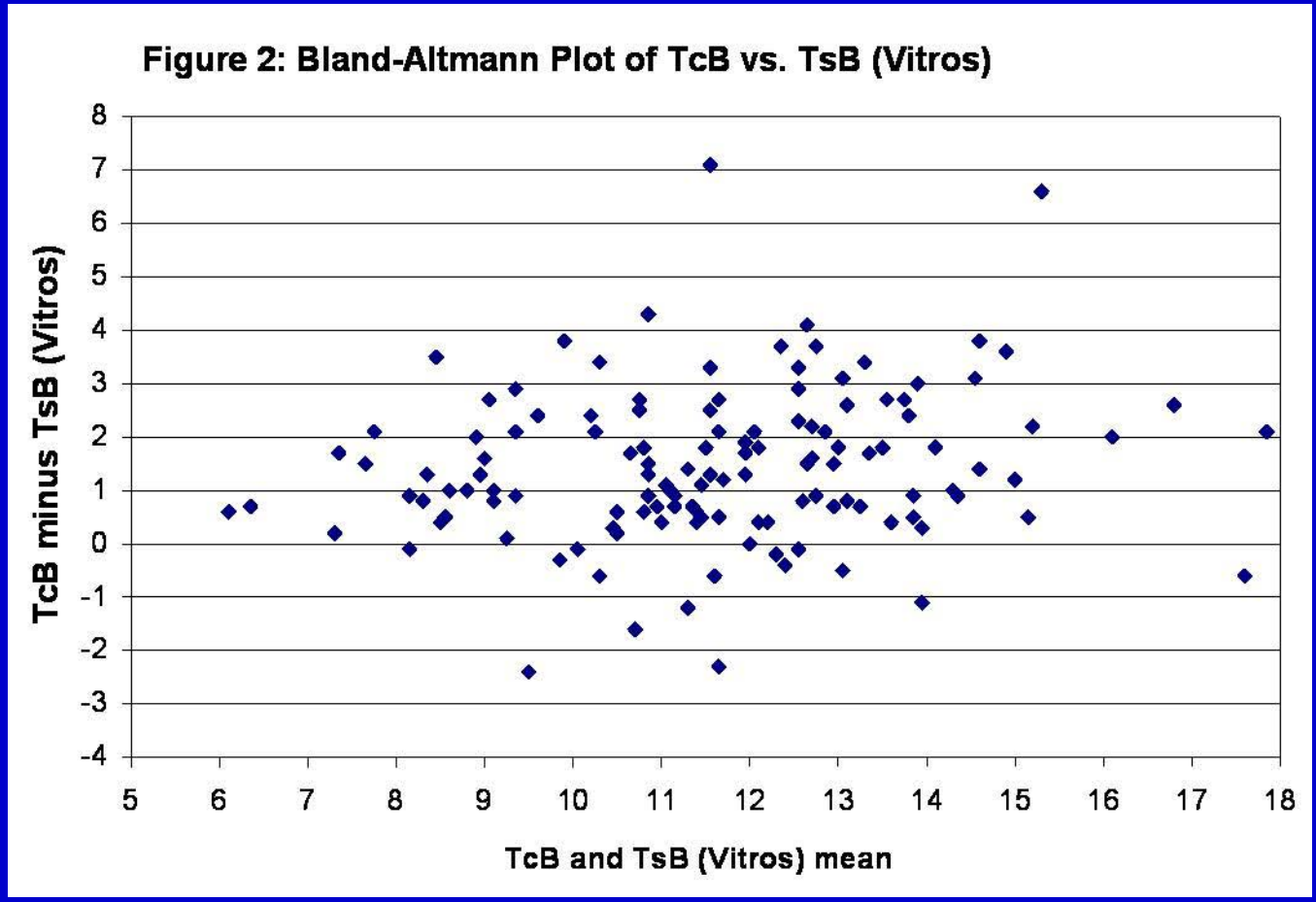


Median bias (TcB minus TsB) = 2.0 mg/dL

Bias relatively constant over range studied

TcB

Results: TcB vs. BuBc TsB



Median bias (TcB minus TsB) = 1.3 mg/dL

Bias relatively constant over range studied

TcB

What is the clinical impact of systematic overestimation of transcutaneous bilirubin?

Can TcB still be used to predict risk of hyperbilirubinemia?

TcB

- Each TcB and TsB value, combined with postnatal age in hours, used to determine risk zone (low, low-intermediate, high-intermediate, high risk) according to Bhutani
- Sensitivity and specificity of high risk TcB for predicting high risk TsB was calculated

TcB

	Transcutaneous bilirubin		
Serum bilirubin (diazo)	Low or low-intermediate risk	High-intermediate or high risk	Total
Low or low-intermediate risk	48	77	125
High-intermediate or high risk	1	51	52
Total	49	128	177

51/52 (98%) sensitivity for predicting high risk diazo TsB

48/125 (38%) specificity for predicting low risk diazo TsB

TcB

	Transcutaneous bilirubin		
Serum bilirubin (Vitros)	Low or low-intermediate risk	High-intermediate or high risk	Total
Low or low-intermediate risk	35	29	64
High-intermediate or high risk	4	63	67
Total	39	92	131

63/67 (94%) sensitivity for predicting high risk BuBc TsB

35/64 (55%) specificity for predicting low risk BuBc TsB

TcB

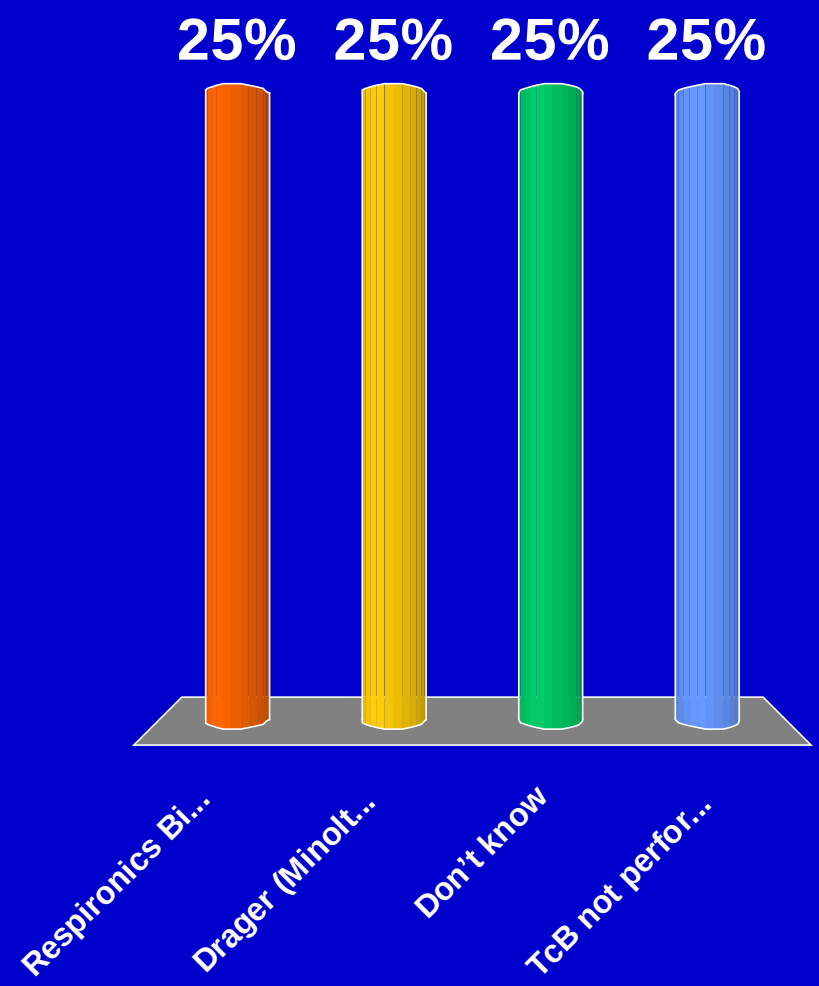
- Rodriguez-Capote et al. Clin Biochem 2009;42:176-9

- Compared 2 TcB methods to BuBc on Vitros
- Both TcB devices underestimated lab bilirubin
- Risk assessment effective only after adjustment for negative bias of TcB
- At Mayo BiliChek overestimated BuBc
- General consensus that TcB screening protocols need to be established institutionally

  Due to variability in lab bilirubin?

Which TcB meter does your facility use?

1. Respironics BiliChek
2. Drager (Minolta) JM 103
3. TcB not performed
4. Don’t know



TcB

- **Unknown variables**

  Variability of TcB compared to lab bilirubin
  - Mayo data: TcB SD at 13 mg/dL ~ 1 mg/dL
  - Compares to lab method SD of ~ 0.2 mg/dL at 20 mg/dL

  Inter-institution variability of TcB?
  - No reference standard for TcB
  - Can only compare to same lab method
  - Use reference method or multiple methods tightly correlated to reference?

  Does universal TcB screening increase treatment intensity?

TcB and reference bilirubin

- What is relationship between TcB and reference method for TSB?

  Roche total bilirubin method recalibrated to better match reference method

  Compare TcB to TSB before and after Roche recalibration

  Compare Roche total serum bilirubin to reference method before and after recalibration using four samples with values assigned by reference method

TcB and reference bilirubin

	Cobas Unit 1		Cobas Unit 2		Reference method performed by Childrens Hospital
	T. Bili	Dir Bili	T. Bili	Dir Bili	
Tube #1	10.7	0.2	11.0	0.2	10.2
	10.5	0.2	10.7	0.2	
Tube #2	13.8	0.2	14.3	0.2	13.08
	13.6	0.2	14	0.2	
Tube #3	18.0	0.2	18.9	0.2	17.06
	17.9	0.2	18.2	0.2	
Tube #4	21.1	0.2	22.1	0.2	19.98
	20.9	0.3	21.5	0.2	

TcB and reference bilirubin

	Cobas Unit 1		Cobas Unit 2		Reference method performed by Childrens Hospital
	T. Bili	Dir Bili	T. Bili	Dir Bili	
Tube #1	9.8	0.2	9.7	0.2	10.2
	9.9	0.2	9.9	0.2	
Tube #2	12.9	0.3	12.9	0.2	13.08
	12.8	0.3	12.8	0.3	
Tube #3	16.9	0.3	17.0	0.3	17.06
	16.9	0.3	16.9	0.3	
Tube #4	19.9	0.3	19.8	0.3	19.98
	19.8	0.3	19.8	0.3	

TcB and reference bilirubin

- Mean (± SD) bias between TcB and TSB for 95 paired TcB/TSB samples before recalibration was 2.5 ± 1.3 mg/dL
- Mean (± SD) bias between TcB and TSB for 118 paired TcB/TSB samples performed after recalibration was 2.9 ± 1.4 mg/dL
- BiliChek TcB overestimates Doumas reference bilirubin by 2.9 mg/dL

TcB and reference bilirubin

- **Future plans:**

  Study relationship between TcB and Roche TSB in multiple facilities, traceability to reference method in all sites

  Determine whether universal TcB screening increases utilization of lab resources (TSB draws) or phototherapy

Conclusions

- Lab bilirubin measurement will vary by method and instrument
- Many lab methods overestimate bilirubin as measured by reference method
- Primary problem is calibration scheme used by vendors

Conclusions

- Relationship between TcB and lab bilirubin primarily function of lab variables
- BiliChek TcB appears to overestimate reference TSB
  - will vary by instrument/institution
- Lack of standardization of lab methods makes inter-lab evaluation of TcB methods difficult
- Each institution must evaluate the effectiveness of TcB screening

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