Issues to consider with neonatal hyperbilirubinaemia

Is your assay good enough?

Prof Patrick Twomey

Questions – hands up

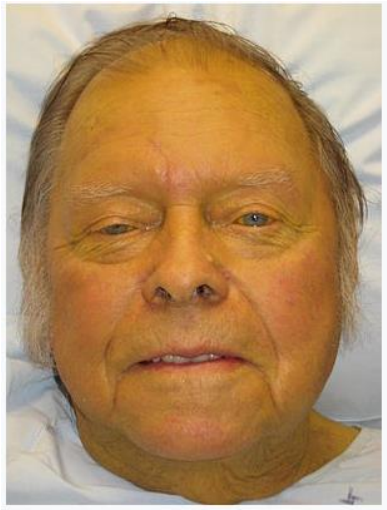
- Who does not serve a neonatal unit?
- Who serves a tertiary (paediatric) liver centre?

Questions – hands up

- Who knows the bilirubin method of their tertiary (paediatric) liver centre?
- How does your bilirubin method compare to the bilirubin method of your tertiary (paediatric) liver centre?

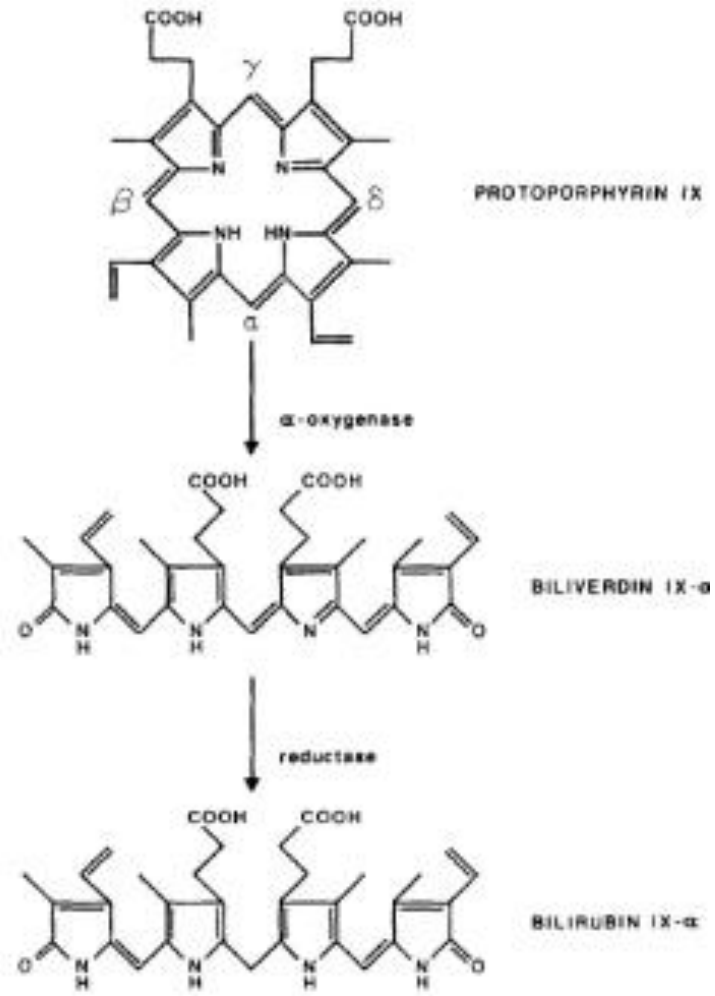
Bilirubin

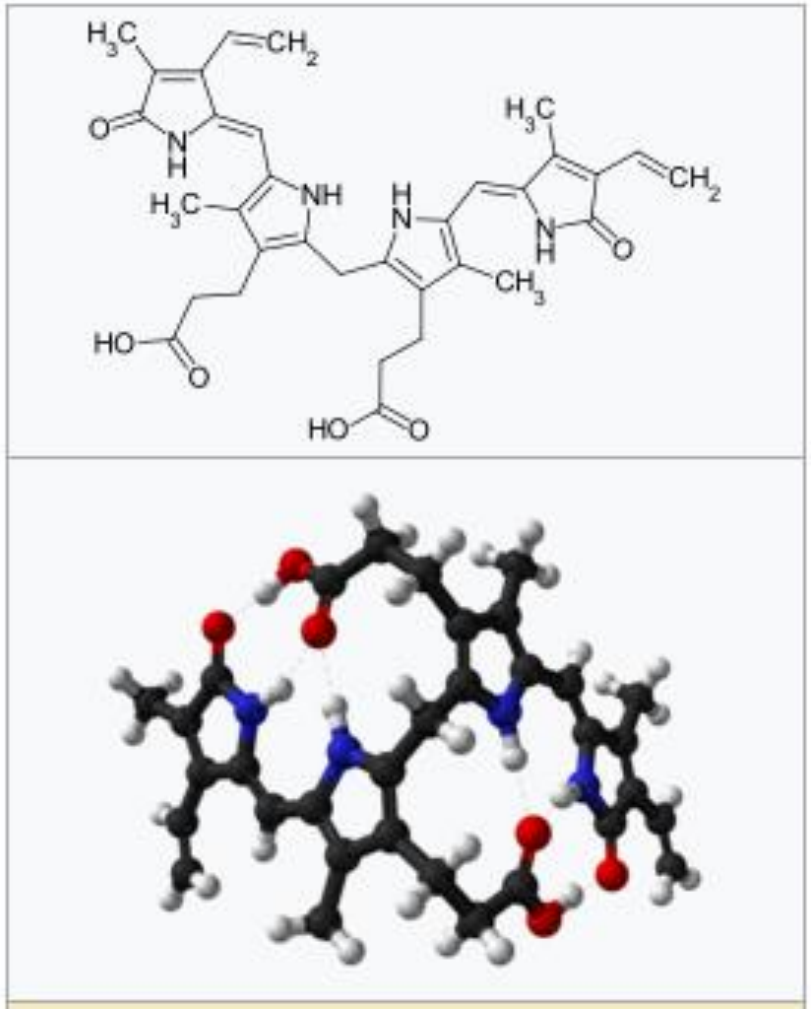
- $C_{33}H_{36}N_4O_6$
- 584.673 g·mol^{-1}
- Hydrophobic and water-insoluble at a pH of 7.4



IUPAC name
3,3′-(2,17-Diethenyl-3,7,13,18-tetramethyl-1,19-dioxo-10,19,21,22,23,24-hexahydro-1*H*-biline-8,12-diyl)dipropanoic acid
Preferred IUPAC name
3,3′-([1^{2}(2)*Z*,6(7^{2})*Z*]-1^{3},7^{4}-Diethenyl-1^{4},3^{3},5^{4},7^{3}-tetramethyl-1^{5},7^{5}-dioxo-1^{1},1^{5},7^{1},7^{5}-tetrahydro-3^{1}*H*,5^{1}*H*-1,7(2),3,5(2,5)-tetrapyrrolaheptaphane-1^{2}(2),6(7^{2})-diene-3^{4},5^{3}-diyl)dipropanoic acid

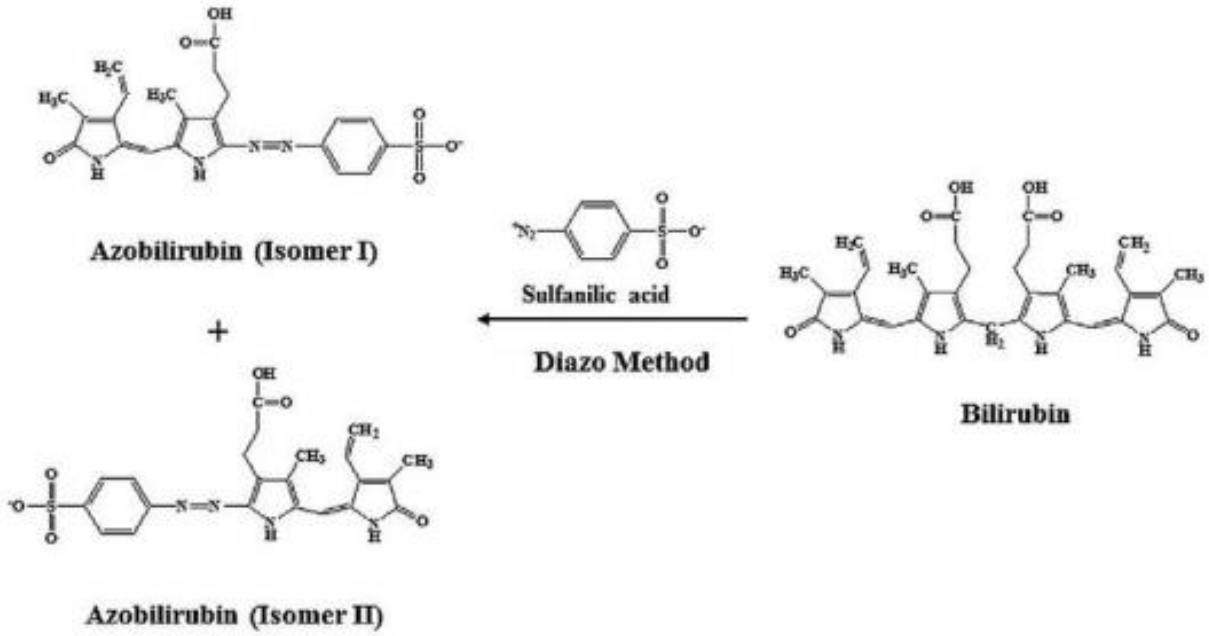
Bilirubin


COOH
COOH
PROTOPORPHYRIN IX
α-oxygenase
BILIVERDIN IX-α
reductase
BILIRUBIN IX-α



Bilirubin assays

- 1883: Paul Ehrlich devised a method of detecting urine bilirubin using diazotised sulfanilic acid to form the red pigment azobilirubin
- 1918: Van den Bergh and Muller first applied this method to serum samples
- Van den Bergh made the novel distinction between direct and indirect bilirubin due to accidentally omitting alcohol
- There have been many modifications to the diazo method over the years



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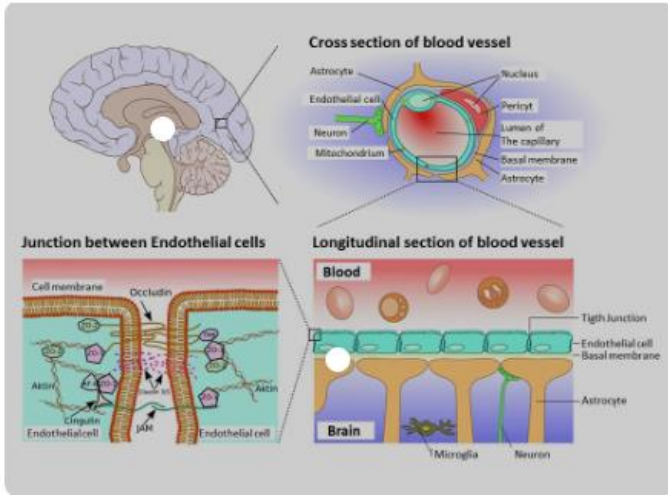
- van den Bergh AAH. Über eine direkte und eine indirekte Diazoreaktion auf Bilirubin 1916

Neonates – are common

- Physiological jaundice is estimated to develop in as many as 50% of term and 80% of preterm neonates
  - the degradation of excess erythrocytes in the first few days of life
  - the immature neonatal liver has reduced activity of the conjugating enzyme glucuronyl transferase, with only around 1% of adult levels
  - Other contributory factors include
    - shorter life span of neonatal erythrocytes (60-90 days in term & 35-50 days in preterm neonates)
    - smaller capillary diameter and
    - birth related bruising

- Accumulation of free hydrophobic unconjugated bilirubin may result in serious clinical sequelae in the new-born as it is capable of permeating across the blood brain barrier, causing injury to neural tissue and long term neurological dysfunction



Neonatal Hyperbilirubinaemia – is common

- 1.2.1 Identify babies as being more likely to develop significant hyperbilirubinaemia if they have any of the following factors:
  - gestational age under 38 weeks
  - a previous sibling with neonatal jaundice requiring phototherapy
  - mother's intention to breastfeed exclusively
  - visible jaundice in the first 24 hours of life.
- 1.2.6 Do not rely on visual inspection alone to estimate the bilirubin level in a baby with suspected jaundice
- 1.2.7 Do not measure bilirubin levels routinely in babies who are not visibly jaundiced

Neonatal Hyperbilirubinaemia

How to measure the bilirubin level

1.2.15 Use serum bilirubin measurement for babies:

- in the first 24 hours of life **or**
- who have a gestational age of less than 35 weeks. **[2016]**

1.2.16 In babies who have a gestational age of 35 weeks or more and who are over 24 hours old:

- use a transcutaneous bilirubinometer to measure the bilirubin level
- if a transcutaneous bilirubinometer is not available, measure the serum bilirubin
- if a transcutaneous bilirubinometer measurement indicates a bilirubin level greater than 250 micromol/litre, measure the serum bilirubin to check the result
- use serum bilirubin measurement if bilirubin levels are at or above the relevant treatment thresholds for their age, and for all subsequent measurements. **[2016]**

1.2.17 Do not use an icterometer to measure bilirubin levels in babies. **[2016]**

Reprinted from THE LANCET, *January* 9, 1960, *pp.* 87–88

A PERSPEX ICTEROMETER FOR NEONATES

I. H. GOSSET
M.A., B.M. Oxon, M.R.C.P.
CONSULTANT PÆDIATRICIAN, NORTHAMPTON GENERAL HOSPITAL

THE icterometer is a simple device for estimating quickly the depth of jaundice in newborn babies at the cotside without taking a blood specimen. The principle is to blanch the baby's skin by pressure, and match the resulting shade of yellow against a colour scale.

The device is valuable as a means of (1) following the depth of jaundice in individual babies from day to day,

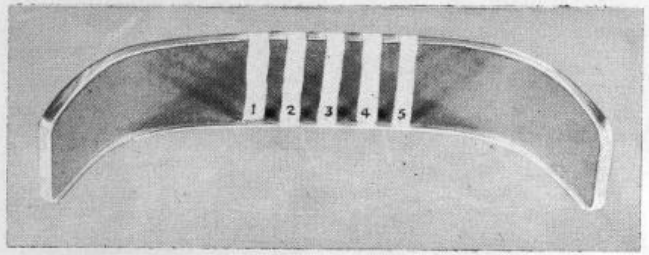
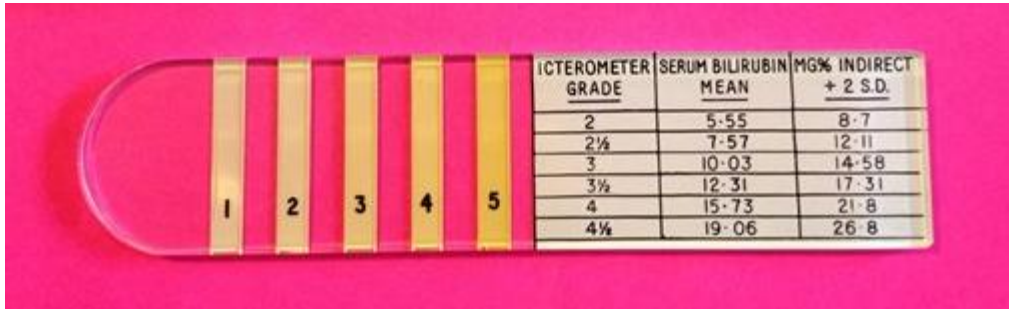


Fig. 1—The icterometer.

and (2) indicating which babies ought to have their serum-bilirubin estimated.



Neonatal Hyperbilirubinaemia

› Acta Paediatr. 2009 Dec;98(12):1909-15. doi: 10.1111/j.1651-2227.2009.01497.x. Epub 2009 Sep 17.

Impact of skin tone on the performance of a transcutaneous jaundice meter

Stephen Wainer [1], Yacov Rabi, Seema M Parmar, Donna Allegro, Martha Lyon

Affiliations + expand

PMID: 19764923 DOI: 10.1111/j.1651-2227.2009.01497.x

Methods: Infants were prospectively categorized into light, medium and dark skin tone groups relative to two reference colours. Transcutaneous bilirubin readings were taken at predetermined intervals through the early neonatal period on a convenience sample of 938 healthy infants > or =37 weeks gestation. Serum bilirubin measurements were drawn routinely with metabolic studies and repeated in the presence of an elevated transcutaneous reading or clinically significant jaundice.

Results: Multivariate linear regression analysis showed a significant impact on serum and transcutaneous bilirubin agreement by skin tone. Highest precision and lowest bias were observed for medium skin toned infants. Greater disagreement between serum and transcutaneous measurements was noted at serum bilirubin concentrations >200 micromol/L. Insufficient numbers of dark skin toned infants were enrolled to evaluate fully the performance of the jaundice meter for this group.

Conclusion: The JM-103 jaundice meter displayed good correlation with serum bilirubin concentrations in light and medium skin tone infants, although it showed a tendency to under-read in the lighter skin tone group and to over-read in the darker skin tone group. The device shows excellent performance characteristics for use as a screening device.

Neonatal Hyperbilirubinaemia

Threshold table

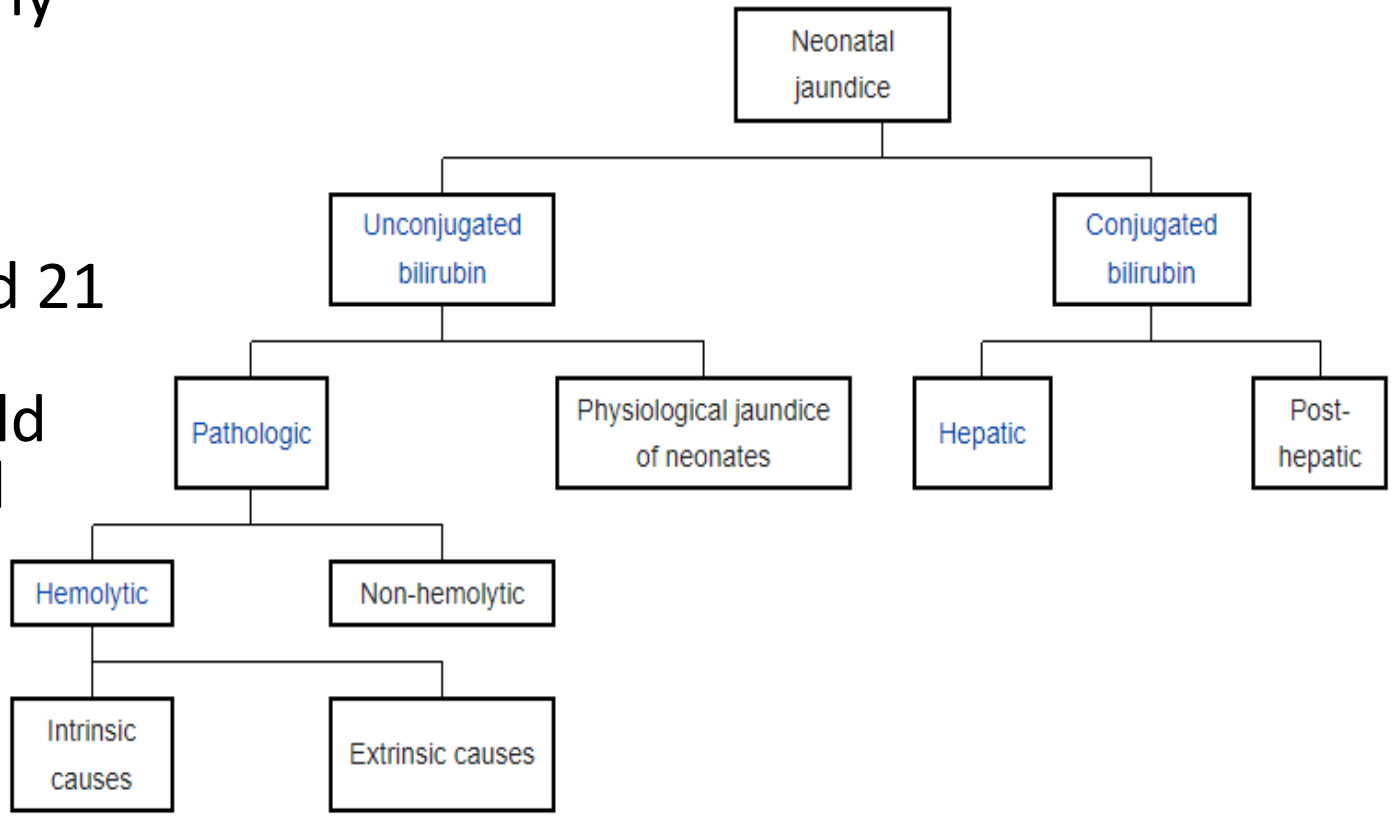
Consensus-based bilirubin thresholds for management of babies 38 weeks or more gestational age with hyperbilirubinaemia

Age (hours)	Bilirubin measurement (micromol/litre)	
0	>100	>100
6	>125	>150
12	>150	>200
18	>175	>250
24	>200	>300
30	>212	>350
36	>225	>400
42	>237	>450
48	>250	>450
54	>262	>450
60	>275	>450

Age (hours)	Bilirubin measurement (micromol/litre)	
66	>287	>450
72	>300	>450
78	>312	>450
84	>325	>450
90	>337	>450
96+	>350	>450
Action	**Start phototherapy**	**Perform an exchange transfusion unless the bilirubin level falls below threshold while the treatment is being prepared**

Neonatal Hyperbilirubinaemia

- Pathological hyperbilirubinaemia should be suspected when jaundice is prolonged or responding poorly to phototherapy
- Any neonate of >37 weeks gestation noted to be jaundiced beyond 14 days of life or beyond 21 days of life in neonates with a gestational age <38 weeks should be evaluated for cholestasis and expert advice should be sought from a neonatal hepatologist.



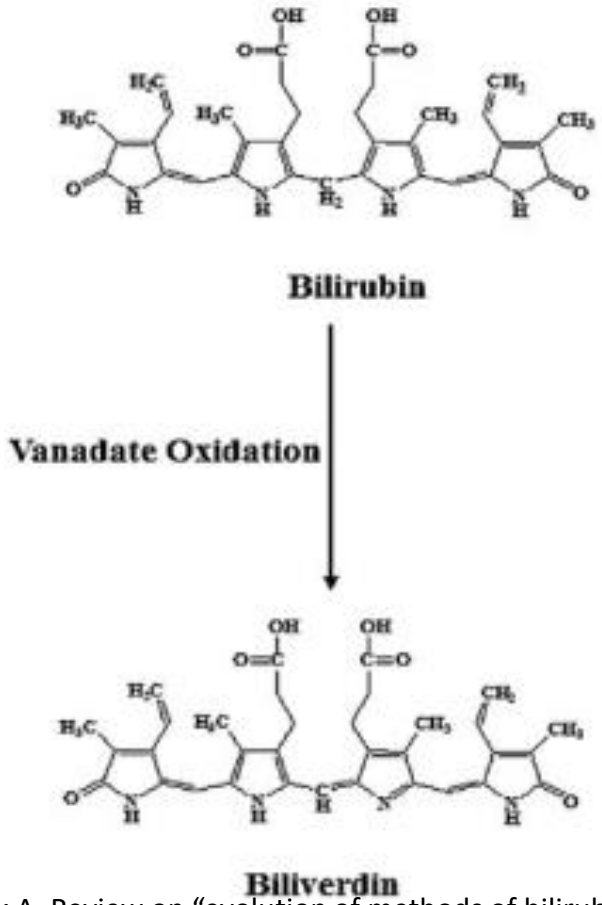
Neonates

- Cholestasis or cholestatic liver disease (CLD) has an incidence of 1 in 2,500 births
- It may be intrahepatic, extrahepatic or both
- Causes may be largely categorised as structural, toxic, metabolic, endocrine or idiopathic

- Biliary atresia is the most common cause of cholestatic jaundice in the first few months of life, accounting for 25%-40% of cases, followed by viral infections and alpha-1 antitrypsin deficiency (α-1ATD) which is the most common inherited aetiology of neonatal chronic liver disease.
- α-1ATD has a relatively high prevalence in Ireland

Bilirubin assays continued

- 1986: Perry *et al* described the first oxidative method for the measurement of total bilirubin
- 1987: Adapted by Doumas *et al* for direct bilirubin. using bilirubin oxidase to oxidise bilirubin to biliverdin, causing a colour change from yellow to purple, then to a colourless product.
  - This method has a superior tolerance to haemolysis interference; however, analyte underestimation is an issue due to incomplete enzymatic oxidation.
- 1991: Patent filed by Wako Pure Chemical Industries Ltd for the manufacture of a commercial kit using a novel method to measure bilirubin using vanadate oxidase as an oxidising agent (discovered by Kuniaki Toduda)
  - This method claims to have superior oxidation capabilities than enzymatic methods, thus improved correlation with conventional methods and to be less effected by interfering molecules including haemoglobin



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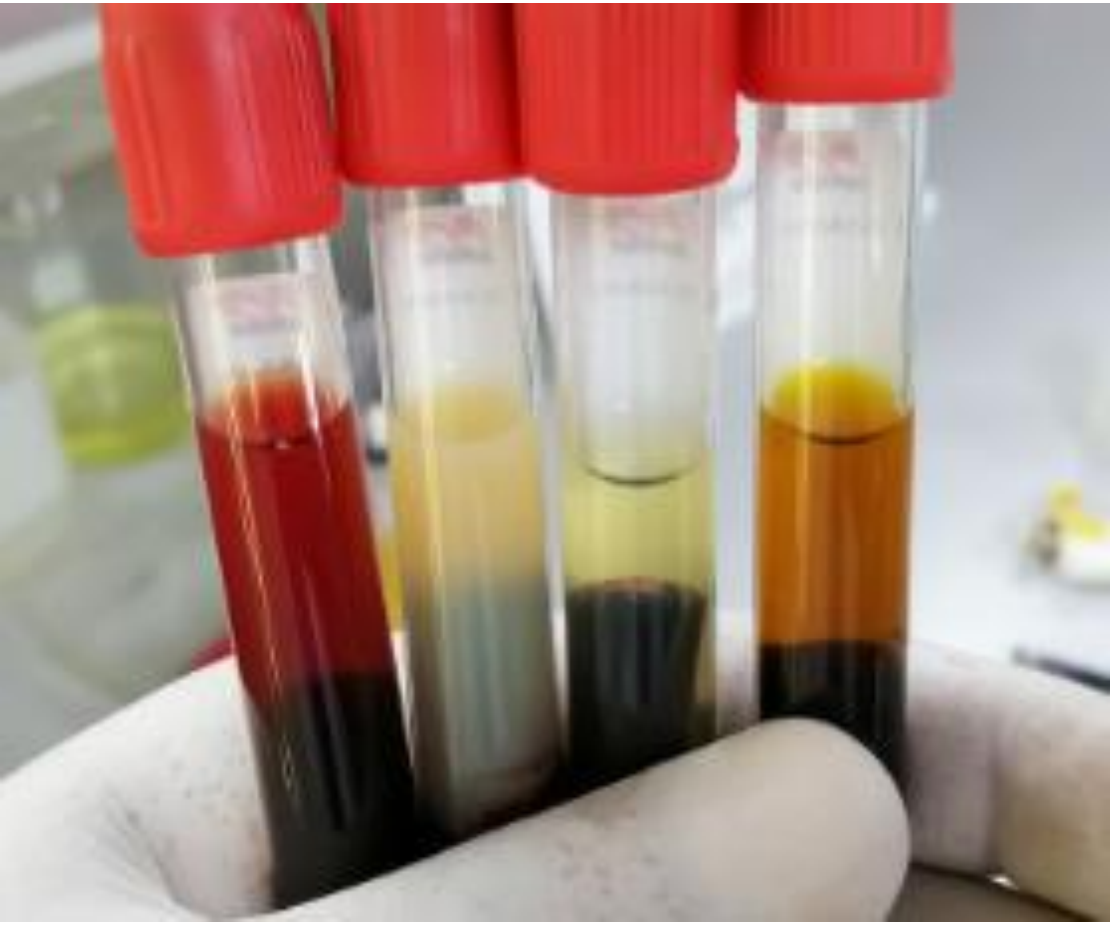
Bilirubin assays continued

- Which method are the NICE (and other) guidance based on?

Haemolysis in general



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Hemolysis, Icterus, and Lipemia Interference: New Approaches to Old Foes

New strategies to resolve three common types of preanalytical interference

PDF VERSION

MICHAEL A. VERA, BA, JOE M. EL-KHOURY, PHD, DABCC, FACB

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In recent years, there has been increased scrutiny of preanalytical errors, which account for as much as 70 percent of all errors in laboratory medicine diagnostic testing. In clinical chemistry testing, a subset of these errors is caused by preanalytical interference, i.e., compounds or molecules that lead to erroneous results by interfering with assays or causing physiological changes to the serum or plasma composition. The most common of these interferences include hemolysis, lipemia, and

Haemolysis in general

Clin Biochem Rev. 2016 Dec; 37(4): 143–151. PMCID: PMC5242478
PMID: 28167844

Current Methods of Haemolysis Detection and Reporting as a Source of Risk to Patient Safety: a Narrative Review

Euan J McCaughey,[1,*] Elia Vecellio,[1,2] Rebecca Lake,[1] Ling Li,[1] Leslie Burnett,[2,3,4] Douglas Chesher,[3,4] Stephen Braye,[3,5] Mark Mackay,[3,6] Stephanie Gay,[6] Tony C Badrick,[6] Johanna I Westbrook,[1] and Andrew Georgiou[1]

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Abstract

Go to: ▸

Aim

Haemolysis has a major impact on patient safety as the need for a replacement specimen increases the risk of injury and infection, delays test results and extends the duration of hospital stays. Consistency of haemolysis detection and reporting can facilitate the generation of benchmark data used to develop quality practices to monitor and reduce this leading cause of pre-analytical laboratory error. This review aims to investigate current methods of haemolysis detection and reporting.

Method

Due to known heterogeneity and immaturity of the research field, a scoping search was conducted using PUBMED, Embase, Medline and CINAHL. Articles published between 2000 and 2014 that reported haemolysis rates in specimens from the general population were included.

Results

Of the 50 studies that met the inclusion criteria, 20 detected haemolysis using the Haemolysis Index (HI), 19 by visual inspection and 13 by undefined methods. There was large intra-study variation in the plasma free haemoglobin level used to establish haemolysis (HI: mean±SD 846±795 mg/L, range 150–3000 mg/L; Visual: 850±436 mg/L, 500–3000 mg/L). Sixteen studies reported the analyte of interest, with only three studies reporting a haemoglobin level at which the specimen would be rejected.

Conclusion

Despite haemolysis being a frequent and costly problem with a negative impact on patient care, there is poor consistency in haemolysis detection and reporting between studies. Improved consistency would facilitate the generation of benchmark data used to create quality practices to monitor and reduce this leading cause of pre-analytical laboratory error.

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

THE ROTUNDA HOSPITAL DUBLIN

A dissertation in partial fulfilment of the Degree of Master of Science in Clinical Chemistry

Trinity College Dublin

Optimisation of the Biochemistry service in the Rotunda Hospital through the introduction of the Wako Direct Bilirubin Assay

By

Ava Brazier

19321021

Dept. Biochemistry

The Rotunda Hospital

Dublin

2021

Haemolysis in neonates

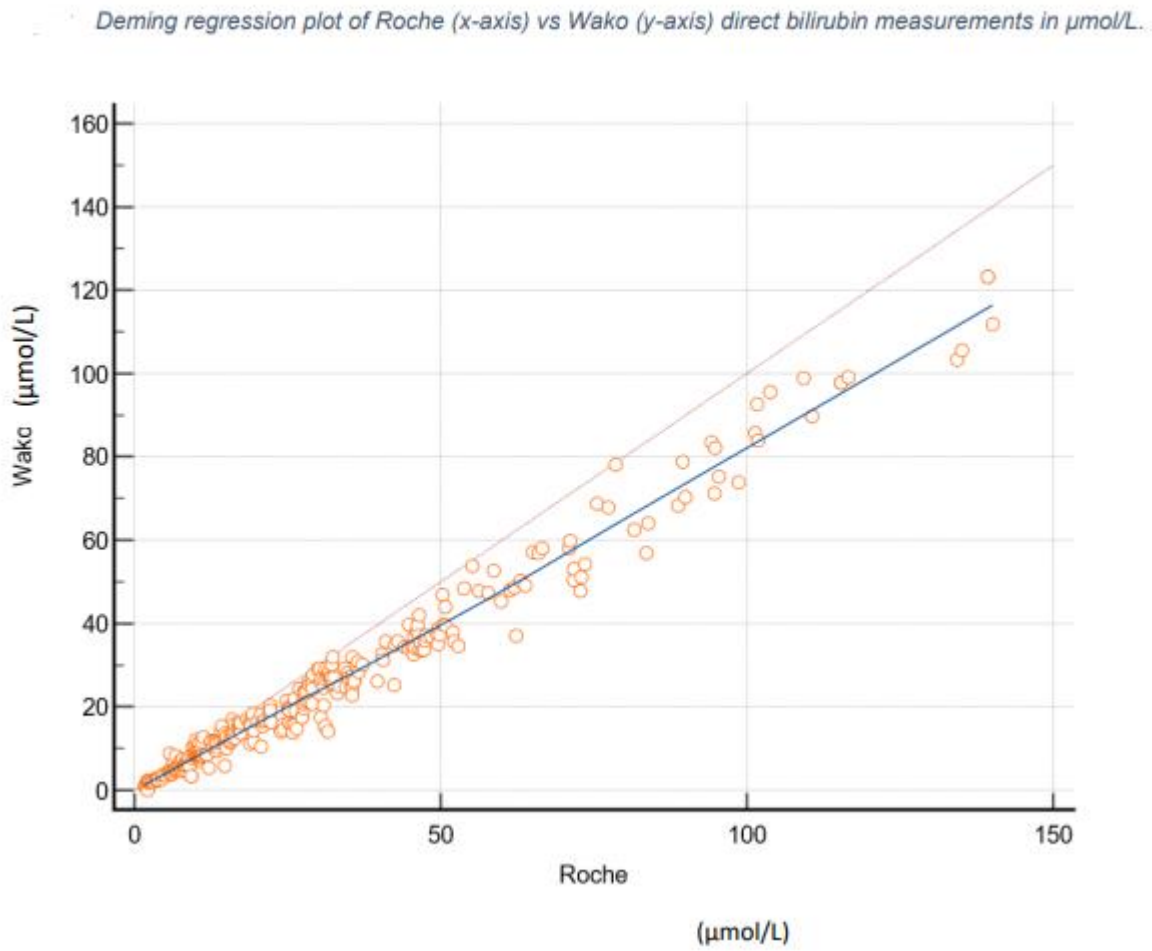
- In 2020, 44% of neonatal samples received were rejected due to haemolysis levels exceeding the manufacturer's stated limit for interference
- The second generation of the Roche direct bilirubin assay (BILD2) – a 2-point end assay which uses the diazo method
- These findings are similar to those observed in the University of Iowa Hospitals and Clinics laboratory which focussed on 47,333 retrospective samples and found that 51.3% of specimens from patients less than 2 years of age exceeded the HI for the Roche direct bilirubin method
- What to do?

- Option 1: to continue current practice of rejecting haemolysed samples for direct bilirubin.
- Option 2: to report quantitative results despite potential interference with a disclaimer to indicate sample is haemolysed. There is a clinical risk involved with this option as inaccurate results may be misleading.
- Option 3 is to explore an alternative method of measurement with a higher limit of haemolysis interference.

Haemolysis in neonates

- Measurement of direct bilirubin by the Wako method is unaffected by interference from haemolysis up to approximate haemoglobin concentration of 310 µmol/L compared to the current Roche threshold of 15.5 (µmol/L)

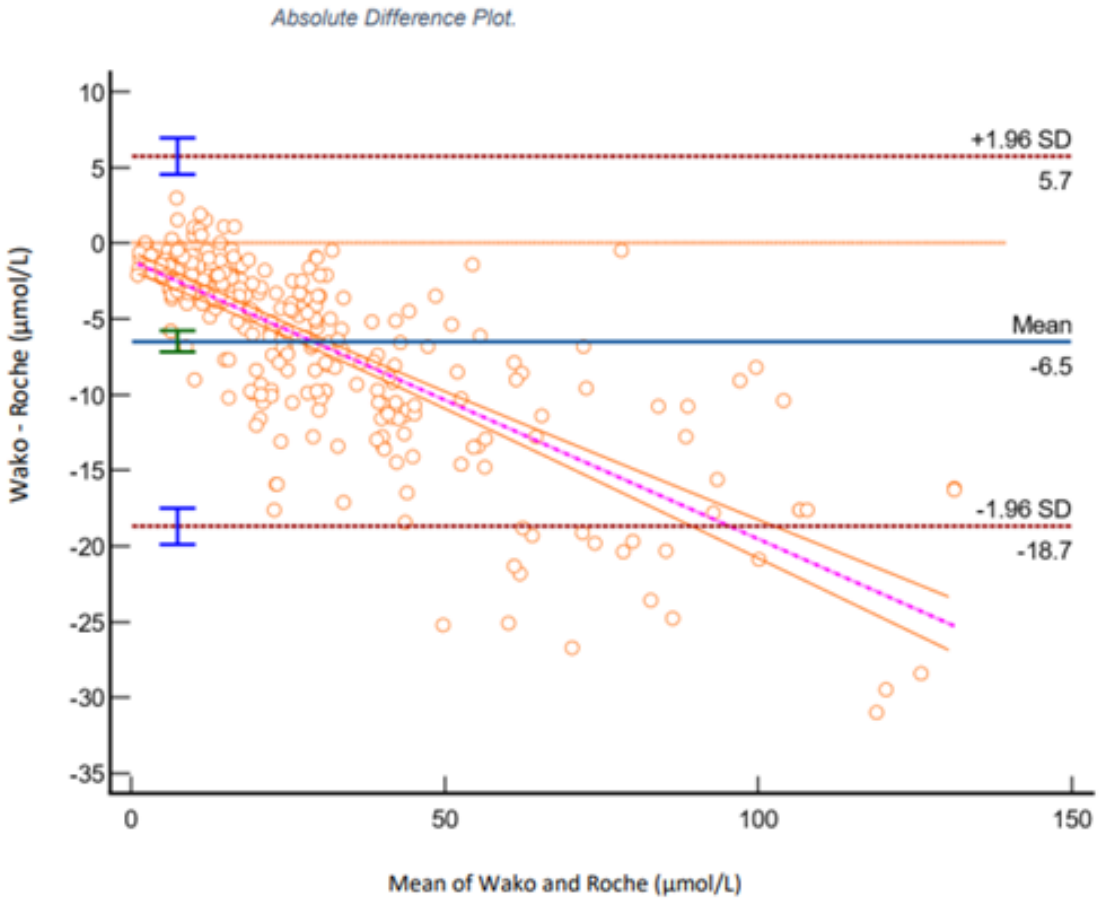
Roche vs. Wako Bilirubin

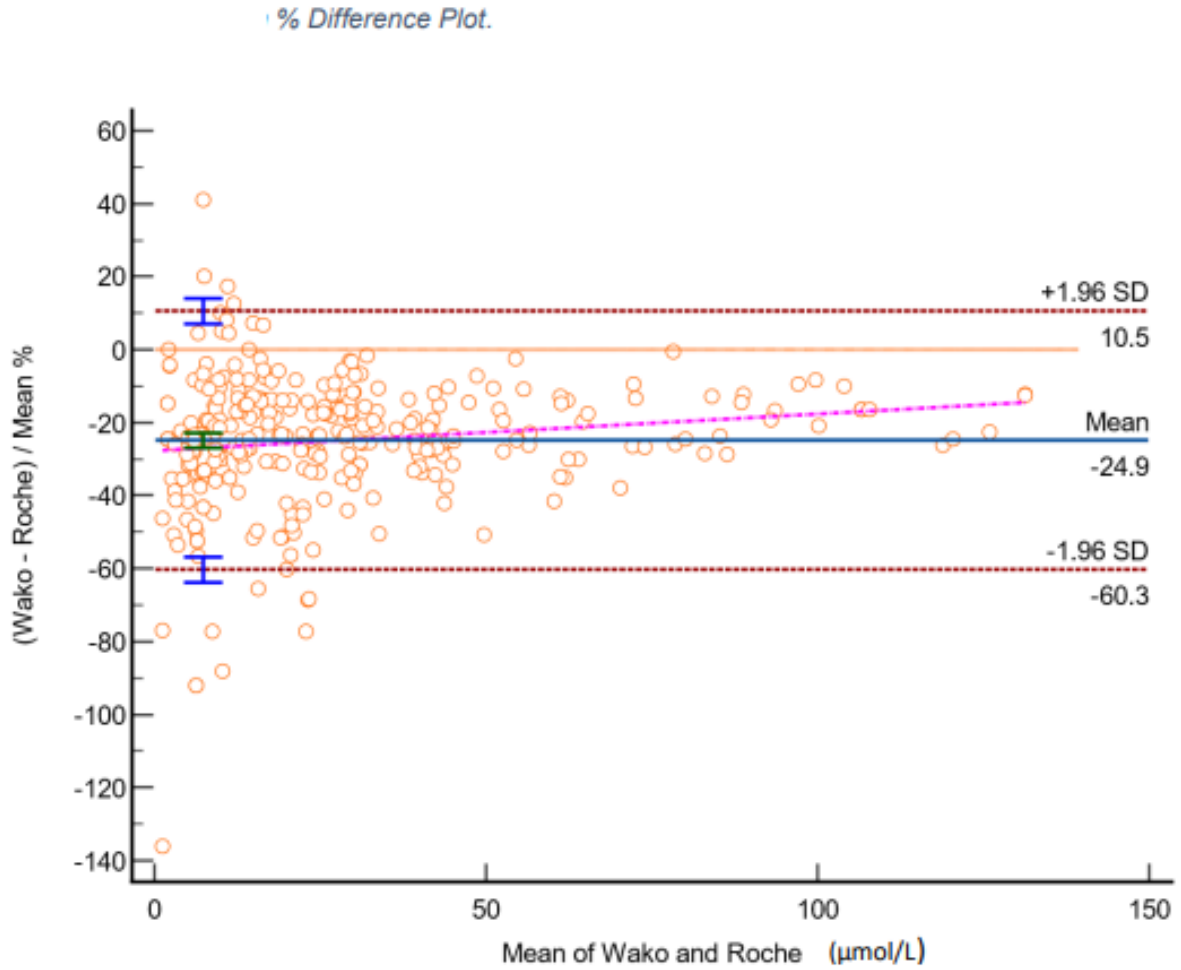


Pearson correlation coefficient	0.9889
95% Confidence interval	0.9861 to 0.9911

Parameter	Coefficient	Std. Error	95% CI
Intercept	-1.1298	0.2921	-1.71 to -0.55
Slope	0.8331	0.01121	0.81 to 0.86

Roche vs. Wako Bilirubin





Roche vs. Wako Bilirubin

- 3081 neonatal specimens were received in the biochemistry laboratory between the 01/01/2020 and 30/06/2020 for direct bilirubin measurement
- 54% had HI values above the limit for rejection (15.5 in µmol/L).
- Had the Wako assay (HI limit of 310 in µmol/L) been in use during this time, the overall rejection rate would have been drastically reduced to 2.53%.
- It had not been decided whether the Wako assay will replace the Roche assay post verification or if it will act as a reflex test for samples exceeding HI values of 15.5 µmol/L
- An obstacle to the immediate replacement of the assay is due to the negative bias of the Wako method compared with the diazo method.
- New reference ranges and action limits would be required

WEQAS

- Total Bilirubin
  - 91 Roche Diazo
  - 51 other platform Diazo
  - 16 other platform Vanadate oxidation

Post-analytical

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Total bilirubin assay differences may cause inconsistent treatment decisions in neonatal hyperbilirubinaemia

David H. Thomas , Janet V. Warner , Graham R.D. Jones , Jason Z.Y. Chung , David J. Macey , Antonella Screnci and Joshua B. Ryan

From the journal Clinical Chemistry and Laboratory Medicine (CCLM)
https://doi.org/10.1515/cclm-2022-0749

This study was initiated following a complaint from a clinician who had transferred a newborn to a tertiary hospital for treatment of severe hyperbilirubinemia but, on arrival, the baby was recategorized into a lower risk category due to a 20% difference in TSB between laboratories. Therefore we investigated whether there are clinically significant method-dependent differences in TSB results from neonatal samples.

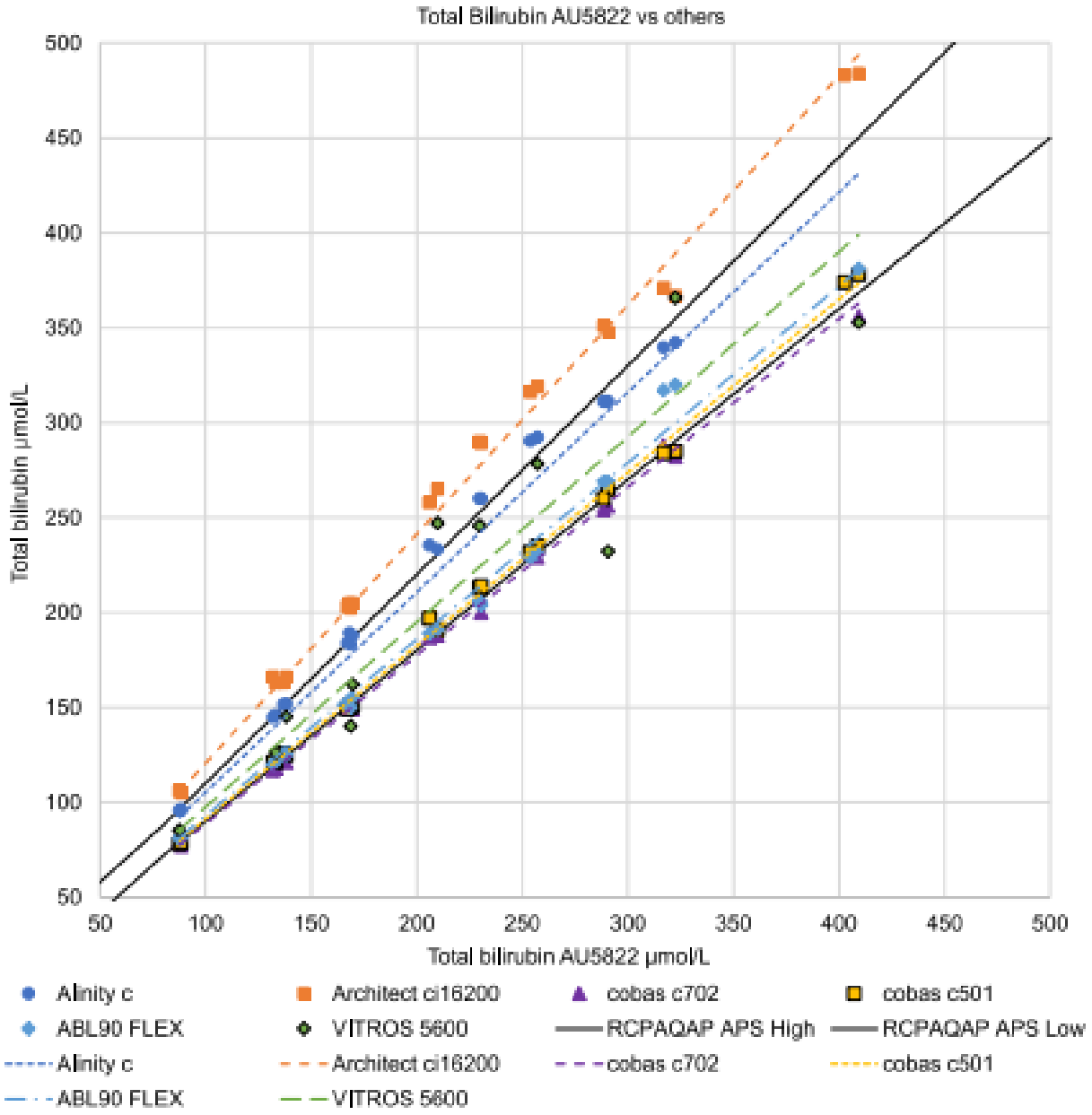

Figure 1: Scattergram of results from each platform with linear trendlines set to pass through the origin.
Solid black lines indicate RCPAQAP high and low analytical performance specifications (+8 µmol/L for results up to 80 µmol/L and +10% for results greater than 80 µmol/L) for the Neonatal Bilirubin program.

(A)

Sample ID	AU5822	Alinity c	Architect ci16200	cobas c702	cobas c501	ABL90 FLEX	VITROS 5600
1	170	188	205	149	151	155	162
2	138	152	166	121	125	125	145
3	169	183	202	149	150	150	140
4	257	292	319	229	235	231	278
5	88	96	106	79	78	81	85
6	322	342	367	282	285	320	366
7	210	233	265	188	191	191	247
8	230	260	290	207	213	206	246
9	290	311	348	256	265	269	232
10	133	146	163	118	121	122	126
11	409	378	484	356	378	381	353
1	168	189	205	151	151	154	
2	137	152	163	123	126	126	
3	167	184	203	150	149	151	
4	254	290	316	231	232	229	
5	89	96	105	77	79	81	
6	317	340	371	288	284	317	
7	206	235	258	187	197	189	
8	230	260	289	200	214	204	
9	289	311	351	254	260	269	
10	132	145	166	117	121	120	
11	403	374	483		374		

Low risk zone	
Low intermediate risk zone	
High intermediate risk zone	
High risk zone	

(B)

Sample ID	AU5822	Alinity c	Architect ci16200	cobas c702	cobas c501	ABL90 FLEX	VITROS 5600
1	170	188	205	149	151	155	162
2	138	152	166	121	125	125	145
3	169	183	202	149	150	150	140
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1	168	189	205	151	151	154	
2	137	152	163	123	126	126	
3	167	184	203	150	149	151	
4	254	290	316	231	232	229	
5	89	96	105	77	79	81	
6	317	340	371	288	284	317	
7	206	235	258	187	197	189	
8	230	260	289	200	214	204	
9	289	311	351	254	260	269	
10	132	145	166	117	121	120	
11	403	374	483		374		

No phototherapy	
Yes phototherapy	

Figure 4: Total bilirubin (µmol/L) results colour coded according to risk zone on Bhutani nomogram at 72 h postnatal age (A) and phototherapy initiation for medium-risk infants (B).

PS

Commutable quality assurance material with analytical performance specifications that are fit for the purpose of assessing and confirming assay performance, especially at the clinical decision points for neonatal jaundice, is also needed

Questions?