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ZIEKENHUIS

H-INDEX VOOR DE BEPALING VAN VRIJ HEMOGLOBINE CRITICALLY APPRAISED TOPIC

MARTIJN LODEWYCKX

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 - › 1.2 H-index
- › 2 Klinische meerwaarde
 - › 2.1 Screening intravasculaire hemolyse
 - › 2.2 ECMO
 - › 2.3 VAD
 - › 2.4 Infecties
 - › 2.5 Andere oorzaken van intravasculaire hemolyse
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 - › 3.1 Accuraatheid
 - › 3.2 Precisie
 - › 3.3 Correlatie tussen verschillende analyzers
- › 4 Invloed van pre-analytiek
- › 5 Kwaliteitsbewaking
- › 6 Conclusie

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1 Inleiding

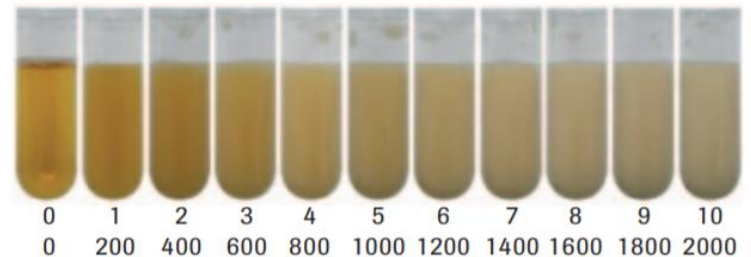
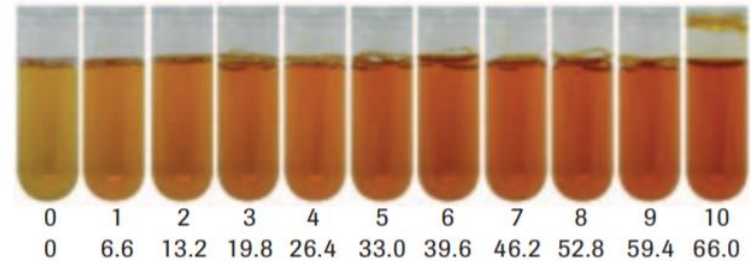
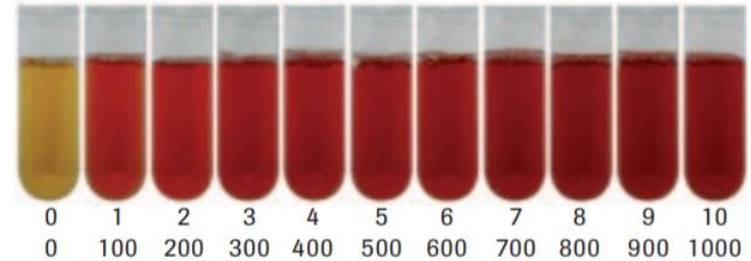
1.1 Serum-indices

> Inschatten mogelijke interferentie

> Hemolyse, icterie, lipemie

> Spectrofotometrie

> (Semi-)kwantitatief of kwalitatief



1 Inleiding

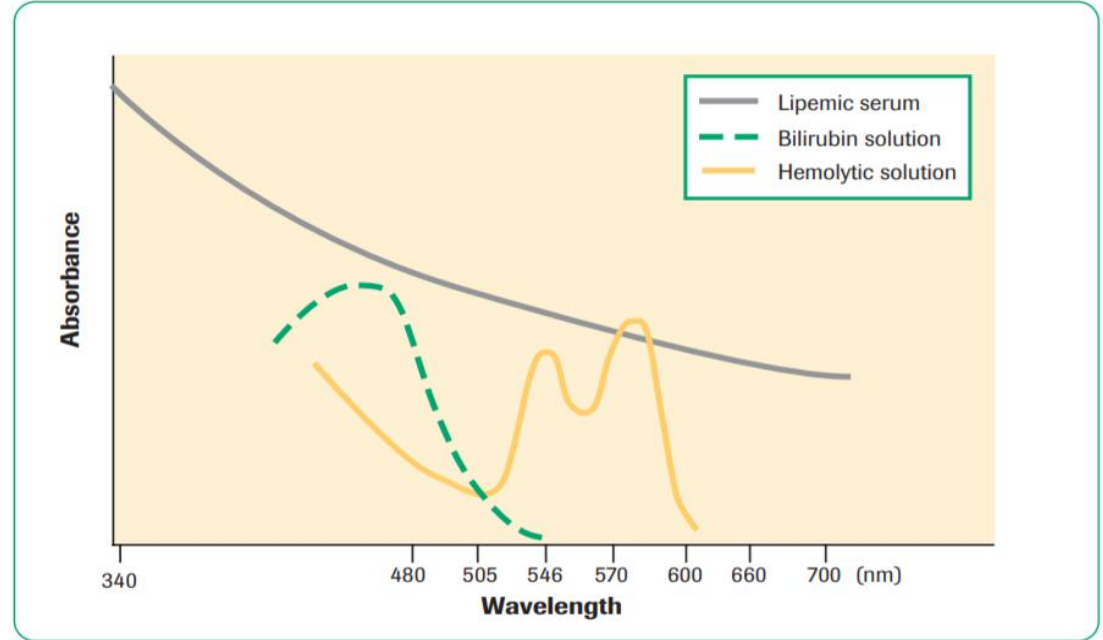
1.2 H-index

› Absorbantiemeting

› Roche: 600/570 nm
(correctie voor lipemie)

› Vrij hemoglobine

› Concrete vraag
intensivist-perfusionist



Roche (2007). Serum Indices: Reduction of clinical errors in laboratory medicine Going straight for the answer. Retrieved from: https://mydialog.roche.com/Htdocs/media/pdf/actualites/2b_SI_Brochure_2007.pdf

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2 Klinische meerwaarde

2.1 Screening intravasculaire hemolyse

› Oorzaken in vivo hemolyse

› Congenitale oorzaken

- › Hemoglobinopathie
- › Deficiënties RBC-membraan
- › Enzymatische defecten

› Verworven oorzaken

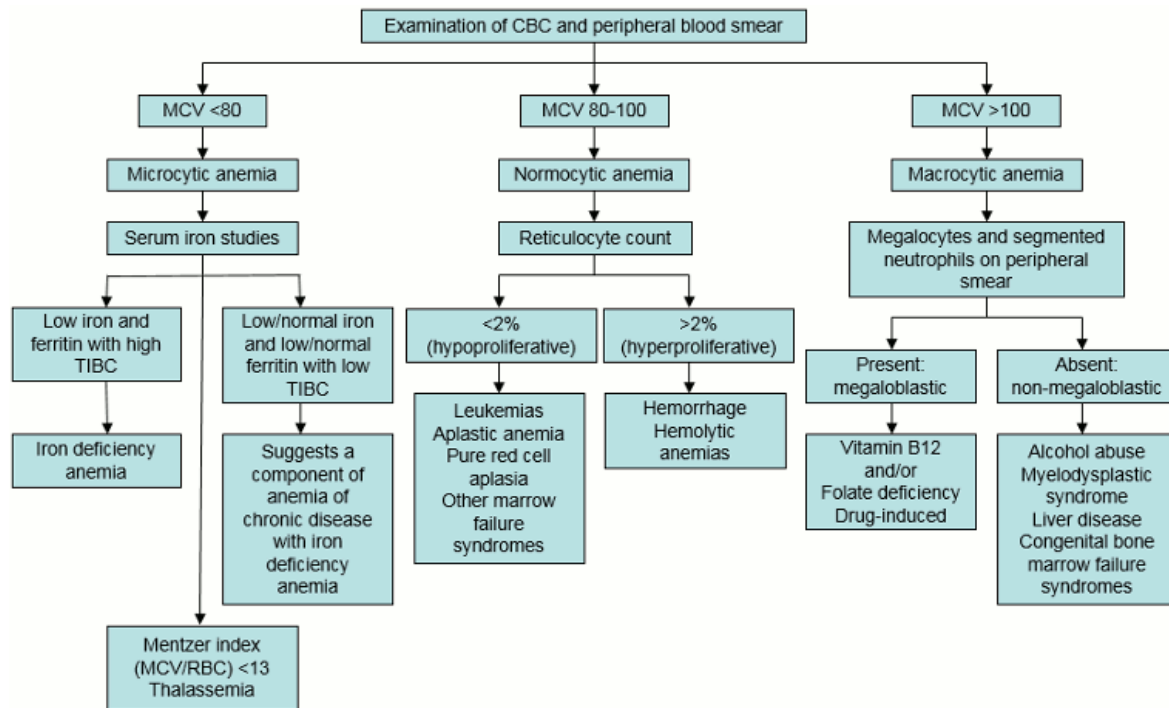
- › Intrinsieke afwijking RBC
- › Mechanische hemolyse
- › Infecties
- › Chemische factoren
- › Immuun-gemedieerd



2 Klinische meerwaarde

2.1 Screening intravasculaire hemolyse

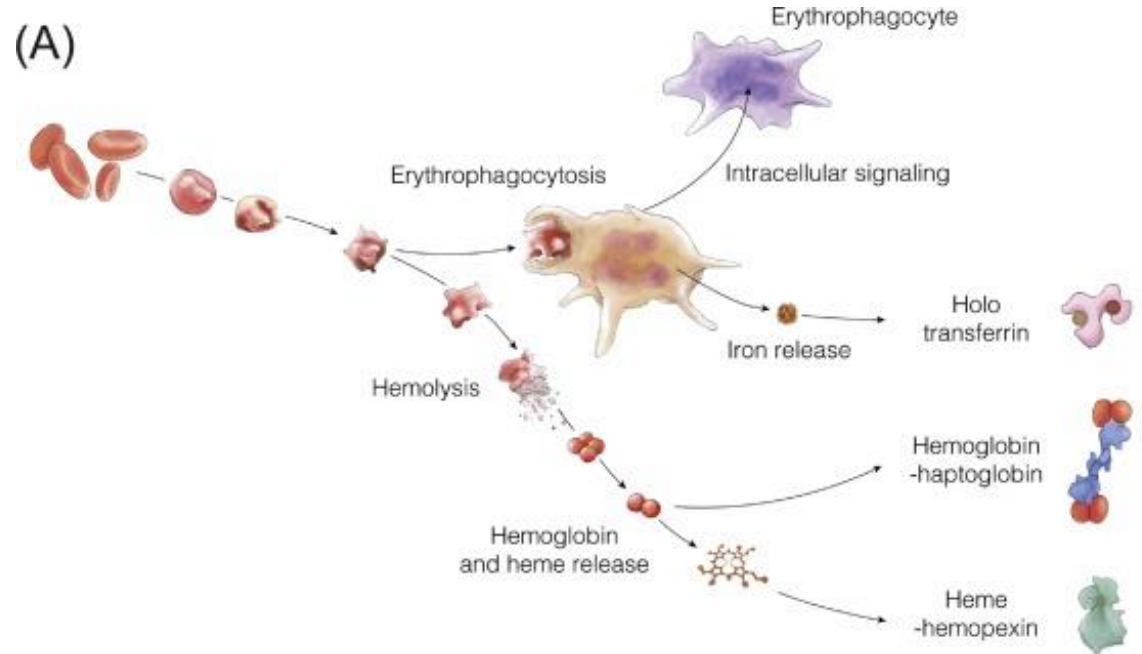
> Diagnostische benadering anemie



2 Klinische meerwaarde

2.1 Screening intravasculaire hemolyse

- › Indirect bilirubine
- › LDH
- › Haptoglobine
- › Vrij hemoglobine?
 - › Weinig specifiek
 - › In vitro – in vivo hemolyse
 - › Niet opgenomen in guidelines



2 Klinische meerwaarde

2.2 Extracorporele membraanoxygenatie

- › Ondersteuning bij ernstig cardiaal of respiratoir falen
- › Trombusvorming
 - › D-dimeren en deltaP
 - › Mechanische hemolyse
 - › Vrij hemoglobine = protrombogeen
- › Nier- en orgaanschade
- › Nood aan transfusie



2 Klinische meerwaarde

2.2 Extracorporele membraanoxygenatie

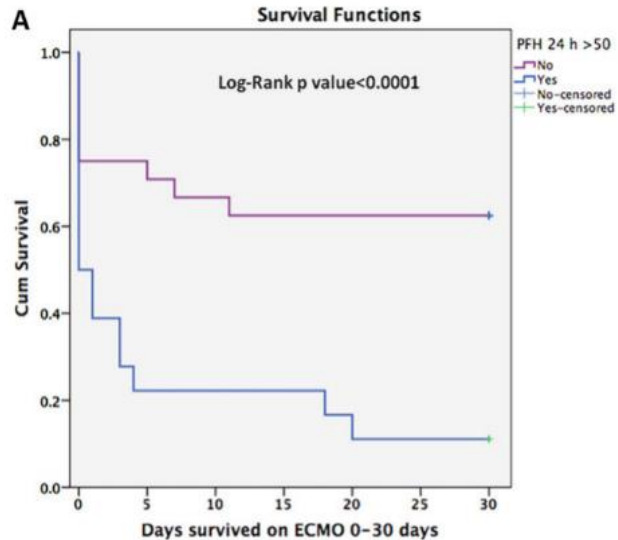


Fig 2. Kaplan-Meier cumulative survival curve comparing subjects with plasma free Hb > 50 mg or < 50 mg/dL checked 24-hour post ECMO initiation for a follow up period of 30 days.

doi:10.1371/journal.pone.0124034.g002

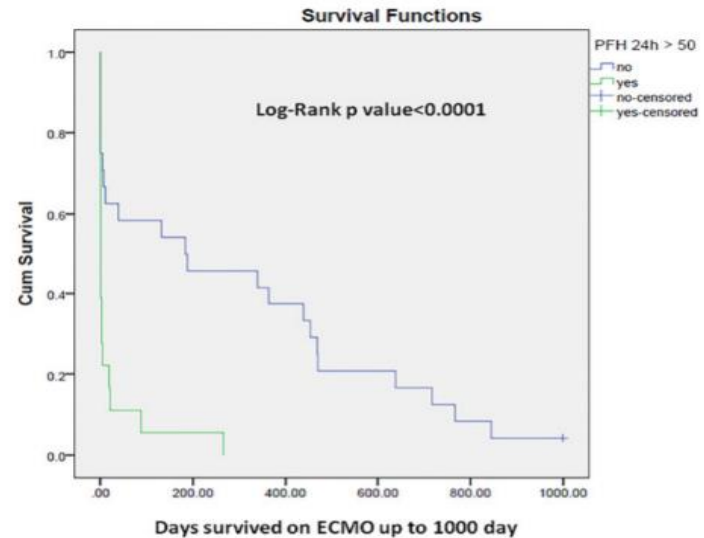


Fig 3. Kaplan-Meier cumulative survival curve comparing subjects with plasma free Hb > 50 mg/dL or < 50 mg/dL checked 24-hour post ECMO initiation up to 3 years of follow up.

doi:10.1371/journal.pone.0124034.g003

Omar, Hesham R; Mirsaedi, Mehdi; Socias, Stephanie; Sprenker, Collin; Caldeira, Christiano; Camporesi, Enrico M; Mangar, Devanand (2015). Plasma Free Hemoglobin Is an Independent Predictor of Mortality among Patients on Extracorporeal Membrane Oxygenation Support. United States: Public Library of Science (PLoS) PLoS one, Vol.10 (4), p.e0124034

2 Klinische meerwaarde

2.2 Extracorporele membraanoxygenatie

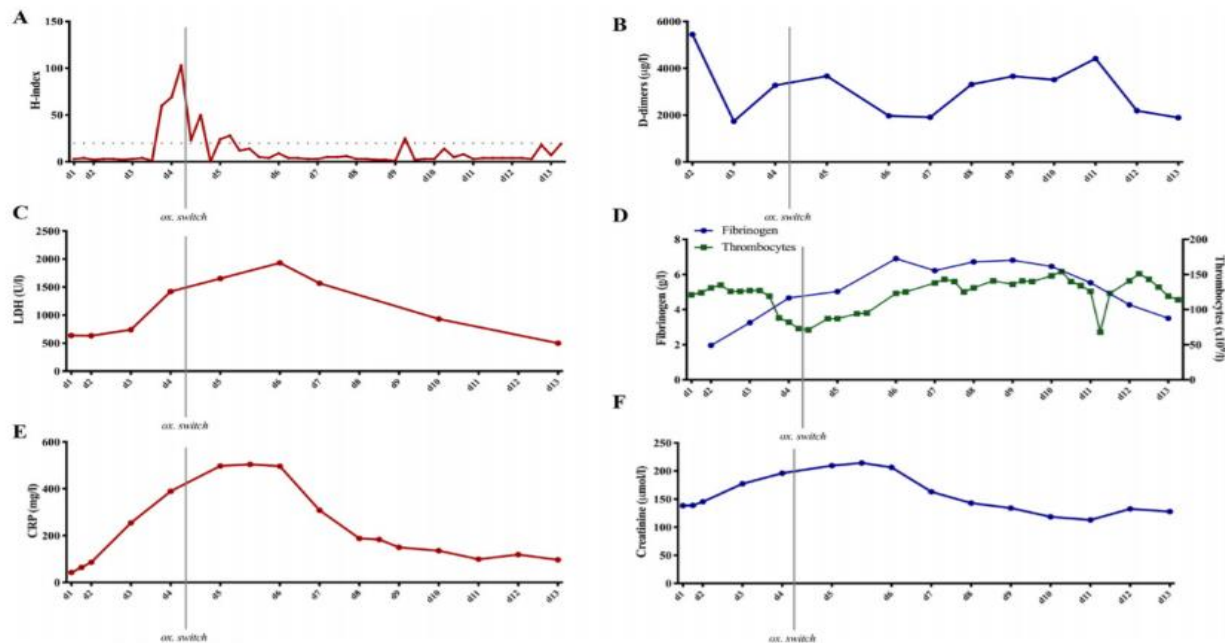


Fig. 3. (A) H-index and (B) D-dimer levels for case 2. (C) LDH levels (ref. <247 U/l). (D) thrombocyte counts and fibrinogen levels (ref. 1.7–3.3 g/l). (E) CRP levels (ref < 10 mg/l). (F) Creatinine levels (ref. 44–80 μmol/l). H-index values were available from all blood analyses (routinely 4 times per day for ECLS patients). The other parameters were measured once per day. D = day, denotes the 7.00 am timepoint of that day. The dotted line indicates the H-index 20 level.

2 Klinische meerwaarde

2.3 Ventriculaire assist devices

- › Mechanische hemolyse
- › Biomarkers: LDH – haptoglobine – vrij hemoglobine
- › Aanpassen flow rate, positie toestel of performance level

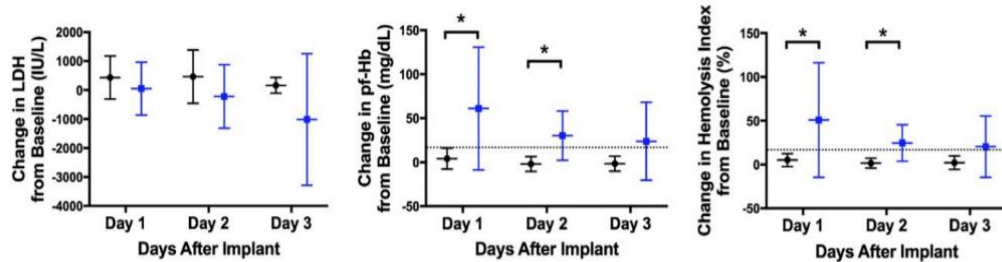


FIG. 3. Change in hemolytic markers within 72 h post-device implant. Compared to patients without hemolysis (black), the change in both pf-Hb and the hemolysis index was significantly higher within 24 h and between 24–48 h among patients with hemolysis (blue). There was no significant difference in the change in LDH levels. * $P < 0.05$ between groups. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

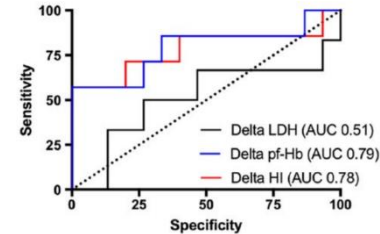


FIG. 4. ROC curves comparing the 24-h change in pf-Hb and LDH from baseline values as a predictor of hemolysis. The C-statistic for the first 24-h change in pf-Hb from baseline was 0.79 ($P = 0.03$) while the C-statistic for a 24-h change in LDH was 0.51 ($P = 0.9$). [Color figure can be viewed at [wileyonlinelibrary.com](#)]

2 Klinische meerwaarde

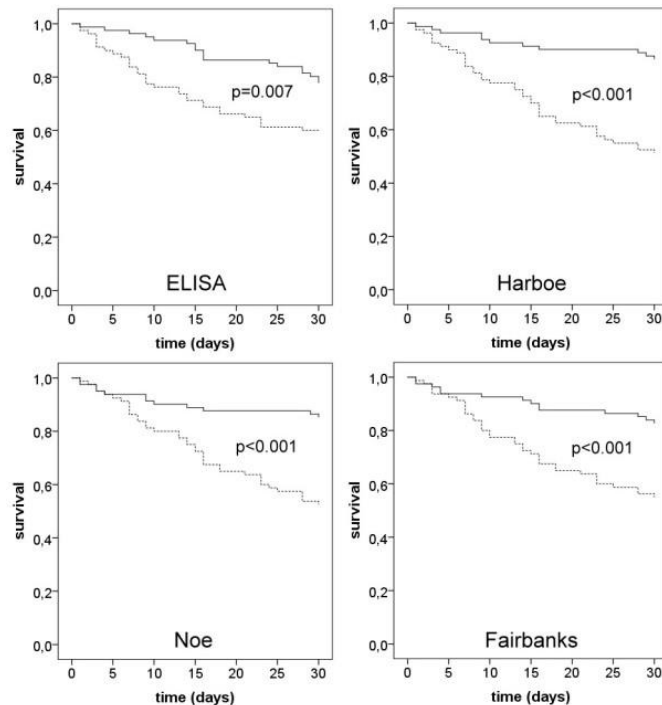
2.4 Infectieuze pathologie

> Sepsis

- > Vrij hemoglobine beïnvloedt inflammatoire respons
- > Hoge concentraties vrij hemoglobine geassocieerd met hogere mortaliteit

> Malaria

- > Vrij hemoglobine veroorzaakt AKI
- > Hoge concentraties vrij hemoglobine geassocieerd met nood aan hemodialyse en hogere mortaliteit



Adamzik, Michael; Hamburger, Tim; Petrat, Frank; Peters, Jürgen; de Groot, Herbert; Hartmann, Matthias (2012). Free hemoglobin concentration in severe sepsis: methods of measurement and prediction of outcome. *Critical care*, Vol.16 (4), p.R125-R125

2 Klinische meerwaarde

2.5 Andere oorzaken van intravasculaire hemolyse

› Vrij hemoglobine toxisch

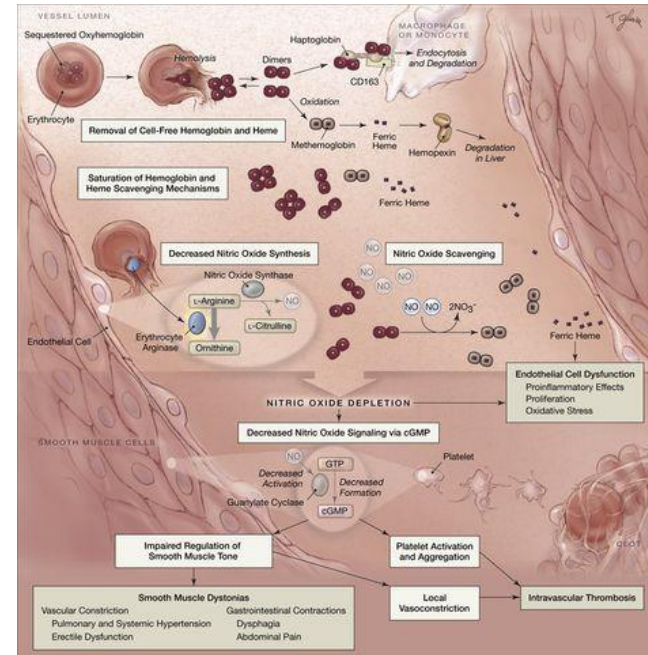
› Consumptie NO

› Vasculaire homeostase

› Plaatjesactivatie en -aggregatie

› Oxidatieve stress

› Nier- en multi-orgaanschade



Rother, Russell P; Bell, Leonard; Hillmen, Peter; Gladwin, Mark T (2005). The Clinical Sequelae of Intravascular Hemolysis and Extracellular Plasma Hemoglobin: A Novel Mechanism of Human Disease. JAMA: the journal of the American Medical Association, Vol.293 (13), p.1653-1662

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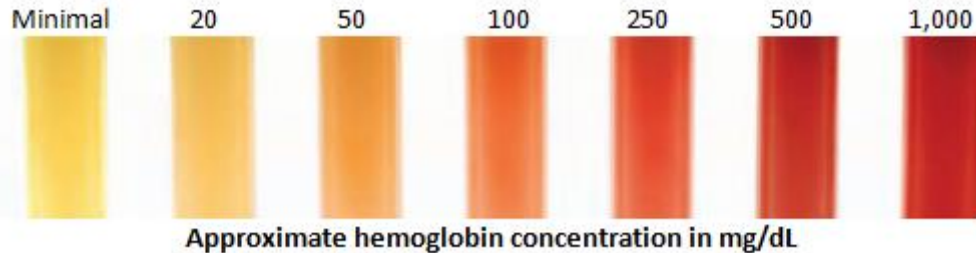
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3 Analytische performantie H-index

3.1 Accuraatheid

- › Visueel
- › Chromogene assays
- › Immunonefelometrische assays
- › Enzyme-linked immunosorbent assays
- › Spectrofotometrische assays
 - › Harboe-methode
 - › Golf-methode
 - › ...



3 Analytische performantie H-index

3.1 Accuraatheid (literatuur)

| Auteurs | Stalen | H-index methode | Vergelijkende assay | Conclusie |
|-------------------------|---|--|--|--|
| Unger et al. (2007) | 200 patiëntstalen | Roche Modular System P | Golf (bichromatische spectrofotometrische assay) | r=0,99 goede correlatie |
| Lippi et al. (2009) | 5 serumstalen, aangerijkt met gehemolyseerd bloed | Roche Modular System P en Integra 400 Plus; Siemens Dimension RxL, ADVIA 2400 en ADVIA 1800; Olympus AU 680 en Coulter DXC 800 | Cyaanmethemoglobine-methode | Modular System P: r=1,00; geen significante bias ADVIA 2400 en 1800: lichte overschatting Andere toestellen: goede correlatie |
| Fernandez et al. (2014) | 6 verdunningen van hemolysaat | Roche Cobas c511, c711, Modular System P; Beckman Coulter 5400, Synchron LXi725; Siemens Advia 2400 en Vista | Cyaanmethemoglobine-methode | Roche Cobas c511, c711 en Modular System P: $\kappa=0,973$ Beckman Coulter Synchron LXi725: $\kappa=0,790$ Siemens ADVIA 2400: $\kappa=0,982$; Siemens Vista: $\kappa=0,821$ |
| Gabaj et al. (2018) | 7 verdunningen van hemolysaat | Cobas 6000 c501 en Abbott Architect c8000 | SLS-methode | Cobas c501: $y = 0,03 (-0,15 - 0,08) + 1,01 (0,98 - 1,03) x$ Architect c8000: $y = 0,02 (0,01 - 0,07) + 1,07 (1,06 - 1,08) x$ |

3 Analytische performantie H-index

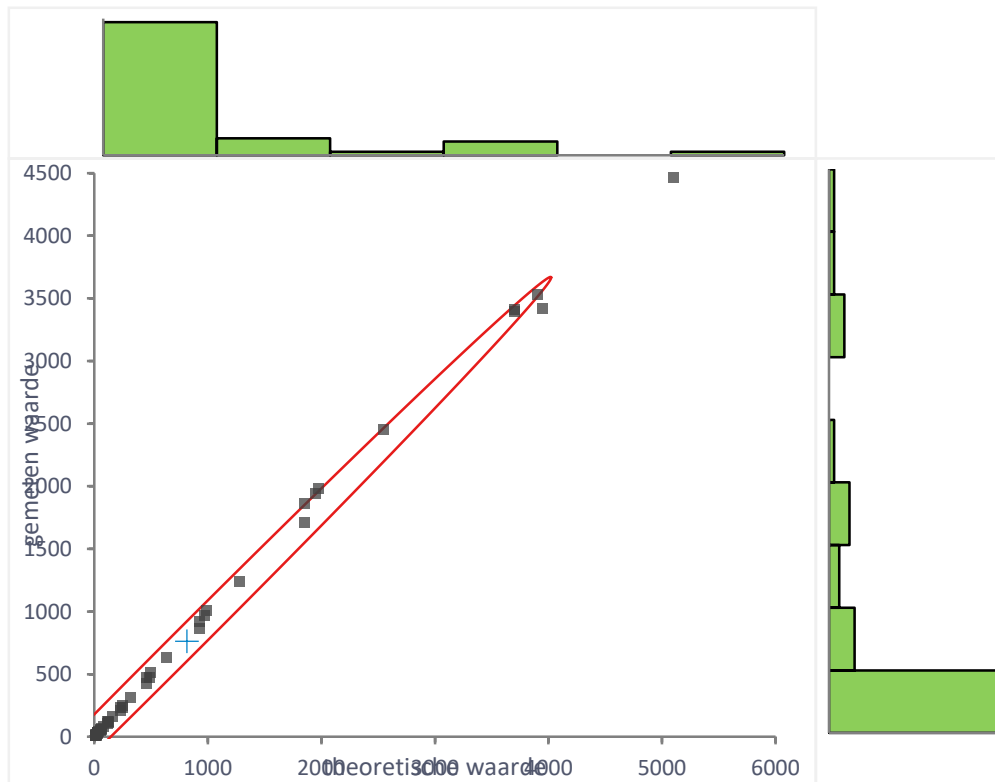
3.1 Accuraatheid (experimenteel)

- › 5 hemolysaten
- › SLS-hemoglobine
methode Sysmex XN-20
- › 5 x 10 verdunningen
- › H-index Roche Cobas
8000 c702 (SI2)



3 Analytische performantie H-index

3.1 Accuraatheid (experimenteel)

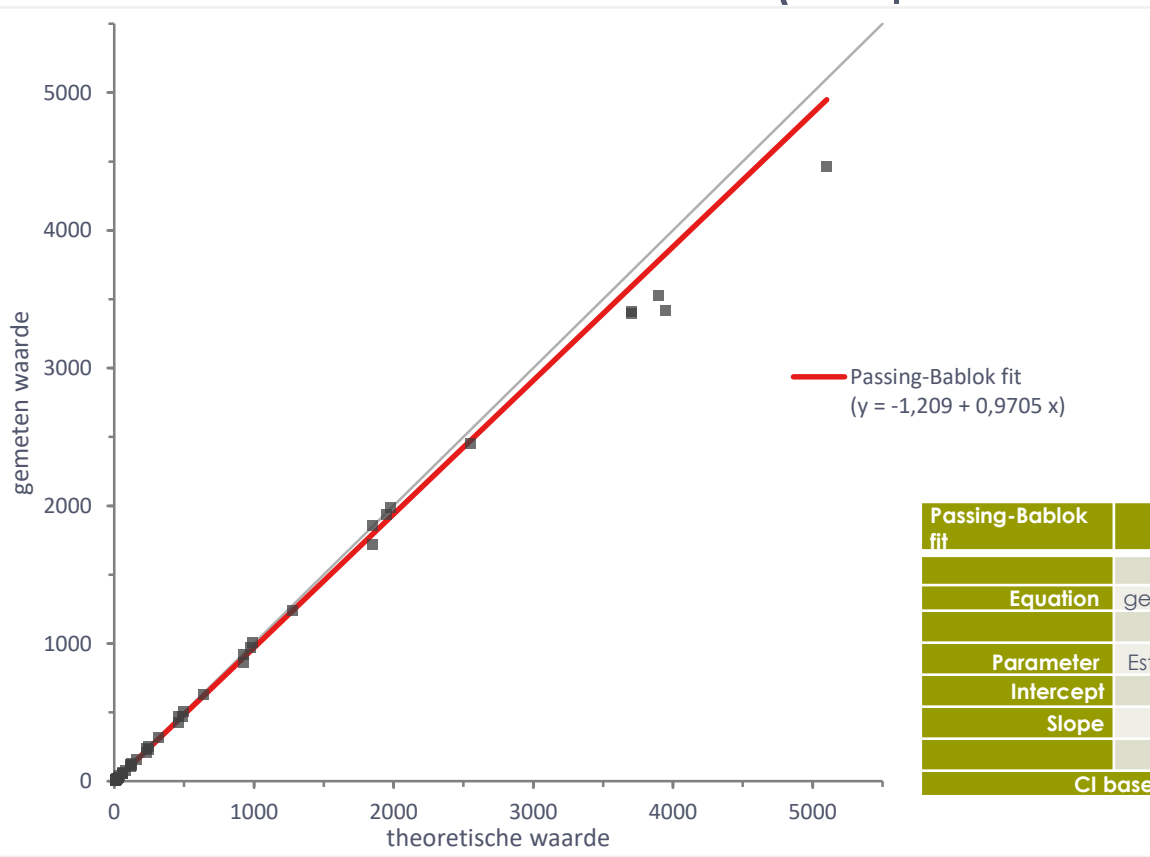


| | | | |
|----------------------|-------|----------|--|
| | | | |
| | | | |
| Pearson's r | 0,998 | | |
| Fisher 95% CI | 0,997 | to 0,999 | |

Correlatie vrij hemoglobine (SLS-methode, Sysmex) – H-index (Roche Cobas c702) (mg/dL).

3 Analytische performantie H-index

3.1 Accuraatheid (experimenteel)



Mediaan verschil: 3,37%

Correlatie vrij hemoglobine (SLS-methode, Sysmex) – H-index (Roche Cobas c702) (mg/dL).

| Passing-Bablok fit | | | | | | | |
|--------------------|--|------------------|-----------|--|--|--|--|
| Equation | gemeten waarde = -1,209 + 0,9705 theoretische waarde | | | | | | |
| Parameter | Estimate | Bootstrap 95% CI | | | | | |
| Intercept | -1,209 | -2,244 | to 0,6590 | | | | |
| Slope | 0,9705 | 0,9267 | to 0,9948 | | | | |

CI based on 999 bootstrap samples.

3 Analytische performantie H-index

3.2 Precisie (literatuur)

| Studie | Analyzer | Intra-run CV (μ : mg/dL) | Within-lab CV (μ : mg/dL) |
|-----------------------|------------------------|--|--|
| Bijsluiter Roche | Roche Cobas c701/c702 | ($\mu = 5$): 27% ($\mu = 127-1116$): 0,7-0,9% | / |
| Gabaj et al. (2018) | Roche Cobas c501 | ($\mu = 30-50$): 1,6% ($\mu = 100-200$): 0,8% | ($\mu = 30-50$): 1,8% ($\mu = 100-200$): 0,9% |
| Gabaj et al. (2018) | Abbott Architect c8000 | ($\mu = 30-50$): 1,7% ($\mu = 100-200$): 0,7% | ($\mu = 30-50$): 2,1% ($\mu = 100-200$): 0,7% |
| Lippi et al. (2018) | Roche Cobas c702 | ($\mu = 101,3$): 1,1% | ($\mu = 87$): 4,9% |
| Reineks et al. (2015) | Roche Cobas c702 | ($\mu = 19$): 5% ($\mu = 295$): 1,1% | / |
| Lippi et al. (2009) | Roche Modular System P | ($\mu = 9-217$): mean CV= 1,2% | / |
| Lippi et al. (2009) | Roche Integra 400 PLUS | ($\mu = 9-217$): mean CV= 2,7% | / |
| Lippi et al. (2009) | Siemens ADVIA 2400 | ($\mu = 9-217$): mean CV= 0,1% | / |
| Lippi et al. (2009) | Siemens ADVIA 1800 | ($\mu = 9-217$): mean CV= 1,0% | / |

3 Analytische performantie H-index

3.2 Precisie (experimenteel)

- › CLSI-EP15-A3 protocol
- › Roche Cobas 6000 c501

| Precisie | Level 1 (mean = 6 mg/dL) | Level 2 (mean = 25 mg/dL) | Level 3 (mean = 135 mg/dL) |
|----------------------------|--------------------------|---------------------------|----------------------------|
| Repeteerbaarheid CV (%) | 14,6 | 3,7 | 2,3 |
| Reproduceerbaarheid CV (%) | 14,3 | 4,8 | 3,3 |

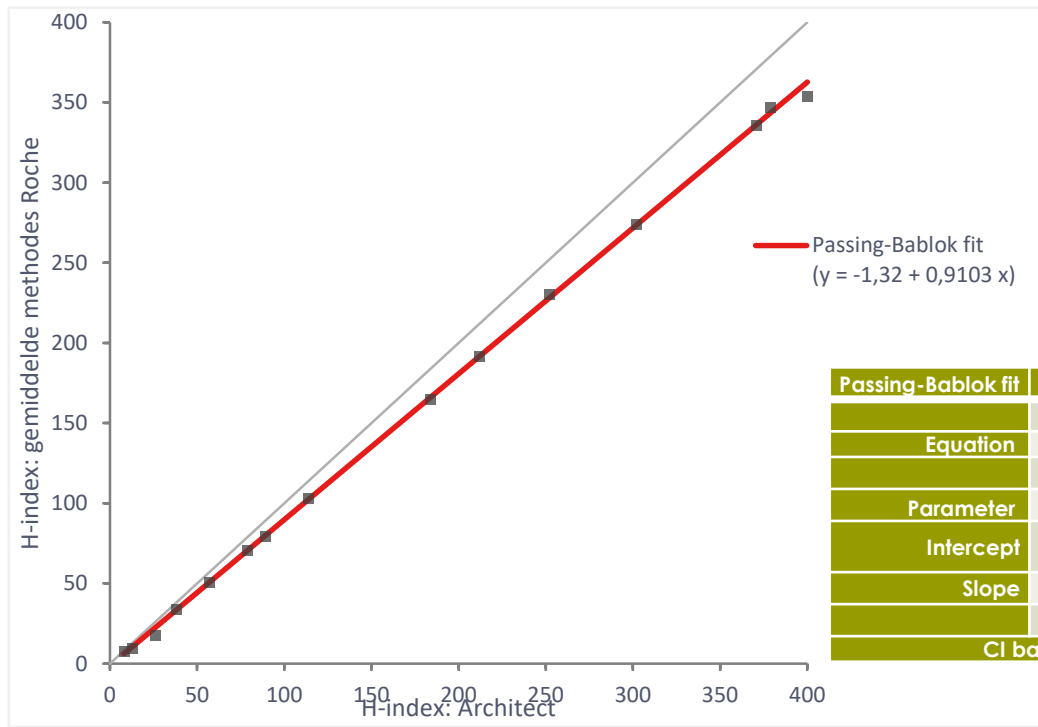
3 Analytische performantie H-index

3.3 Correlatie verschillende toestellen (literatuur)

| Studie | Analyzers | Overeenkomst |
|--------------------------------|--|---|
| Bijsluiters Roche | C501, c503, c702 | Goede correlatie: $r > 0,995$, geen significante bias |
| Gabaj et al. (2018) | Abbott Architect c8000, Roche Cobas c501 | (Cobas = 0,02 (0,01-0,02) + 0,87 (0,86-0,87) Architect) |
| Fernandez et al. (2014) | Roche Cobas c511, c711, Modular System P; Beckman Coulter 5400, Synchron LXi725; Siemens Advia 2400 en Vista | Goede correlatie (kwalitatieve vergelijking) |

3 Analytische performantie H-index

3.3 Correlatie verschillende toestellen (experimenteel)



| Passing-Bablok fit | | | |
|------------------------------------|--|------------------|------------|
| Equation | H-index: gemiddelde methodes Roche = -1,32 + 0,9103 H-index: Architect | | |
| Parameter | Estimate | Bootstrap 95% CI | |
| Intercept | -1,320 | -3,533 | to 0,08617 |
| Slope | 0,9103 | 0,8954 | to 0,9231 |
| CI based on 999 bootstrap samples. | | | |

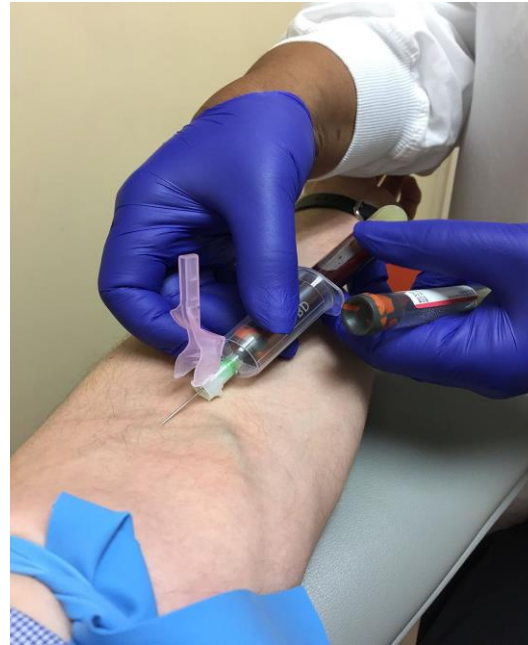
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4 Invloed van pre-analytiek

- › “In vitro” hemolyse
 - › Bloedafname
 - › Type naald
 - › Extractiesysteem
 - › Type vasculair access
 - › Vasculaire status van patiënt
 - › Transport
 - › Duur
 - › Temperatuur
 - › Type
 - › Staalvoorbereiding
 - › Centrifugatie



4 Invloed van pre-analytiek

| | n | % |
|------------------------------------|-----|------|
| Blood drawn too vigorously through | | |
| Needle into syringe | 155 | 30.7 |
| Butterfly needle into syringe | 101 | 20 |
| Intravenous catheter into syringe | 83 | 16.5 |
| Infusion access into syringe | 58 | 11.5 |
| Catheter partially obstructed | 35 | 6.9 |
| Blood forced into the tube | 26 | 5.1 |
| In vivo hemolysis | 9 | 1.8 |
| Extracorporeal circulation | 7 | 1.4 |
| Specimen frozen | 4 | 0.8 |
| Errors in handling | 1 | 0.2 |
| Cause unknown | 26 | 5.1 |
| Total | 505 | 100 |

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5 Kwaliteitsbewaking

- › CLSI: kwaliteitsbewaking nodig
 - › Accepteren of verwerpen stalen
 - › Waarschuwen voor interferentie
- › Spectrofotometrische bepaling gevoelig aan drifts en fouten
- › Geen kalibratoren beschikbaar
 - › Roche: enkel blanco-kalibratie
- › Geen eQC van WIV
- › Wel commerciële controlematerialen



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6 conclusie

} +

- } Accurate en precieze bepaling van vrij hemoglobine
- } Klinische meerwaarde in specifieke settings

} -

- } In vitro hemolyse
- } Validatie van methode en kwaliteitsbewaking nodig

A young child with blonde hair, wearing a yellow jacket and dark boots, is sitting on a grey bench in a hospital waiting area. The child is focused on playing with a colorful wooden toy box. The toy box is decorated with various sea creatures and patterns. On top of the box is a wooden table with several colorful beads and a complex structure of colorful rings and rods. A woman in a pink shirt is leaning over the table, looking at the child's play. The background features a large mural of an underwater scene with various sea creatures like jellyfish, fish, and coral. The wall is blue and white, and there are windows in the background.

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EINDE