

# Total Haemoglobin – implementation of a reference system

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*JCTLM – Members' and Stakeholders' meeting  
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# Status

## JCTLM database – listings:

### List of reference measurement methods/procedures

This file was created on 15 November 2019 from the JCTLM-DB website (<https://www.bipm.org/jctlm/>)

Your search criteria: Reference measurement methods/procedures; Analyte: hemoglobin; Analyte category: -; Matrix category: -

#### Spectrophotometry method for hemoglobin in whole blood

- ▶ Cyanmethemoglobin

Applicable matrix(s)	whole blood
Full description of technique(s)	Spectroscopy after reaction with KCN
Quantity	
Applicable range	
Expected uncertainty	
Reference(s)	NRSCL H15-A3
JCTLM DB identification number	C1RMP_P18

# Status

- **1960s: Standardization**

Zijlstra W G, Kampen E J, Standardization of haemoglobinometry, I. The extinction coefficient of hemoglobincyanide. Clin Chim Acta **1960**;5:719-26.

- **WHO Standard material**

The material is derived from lysed bovine blood converted to hemoglobincyanide (HiCN);  
Davis B H, Jungerius B, International Council for Standardization in Haematology technical report 1-2009: new reference material for haemoglobincyanide for use in standardization of blood haemoglobin measurements. Int Jnl Lab Hem **2010**;32:139-41.

- **CLSI Standard**

H15-A3  
Vol. 20 No. 28

Replaces H15-A2  
Vol. 14 No. 6

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Reference and Selected Procedures for the Quantitative Determination  
of Hemoglobin in Blood; Approved Standard—Third Edition

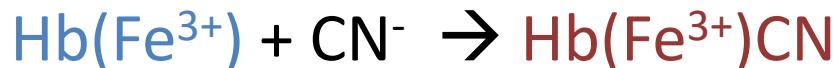
# HiCN – Analytical principle

- Hemolysation

- Oxidation:



- HiCN building:



HiCN =  
Hemoglobincyanide =  
Cyanmethaemoglobin

# HiCN – Analytical principle

	Standards				
Parameter	DIN German Institute for Standardization	CLSI Clinical & Laboratory Standards Institute	BSI British Standards Institution	ICSH Int. Council of Standardization in Hematology	Reference measuring System
K <sub>3</sub> [Fe(CN) <sub>6</sub> ] conc.	0.61 - 1.0 mM	0.607 mM	0.607 mM	0.607 mM	0.60 mM
Cyanide conc.	0.8 - 2.0 mM	0.768 mM	0.768 mM	0.768 mM	1.00 mM
Buffer conc.	Required, e.g. NaH <sub>2</sub> PO <sub>4</sub> ; conc. not defined	1.029 mM KH <sub>2</sub> PO <sub>4</sub> = 140 mg/L ≡ 160 mg/L NaH <sub>2</sub> PO <sub>4</sub>	1.029 mM KH <sub>2</sub> PO <sub>4</sub> = 140 mg/L ≡ 160 mg/L NaH <sub>2</sub> PO <sub>4</sub>	1.029 mM KH <sub>2</sub> PO <sub>4</sub> = 140 mg/L ≡ 160 mg/L NaH <sub>2</sub> PO <sub>4</sub>	2.50 mM Na <sub>2</sub> HPO <sub>4</sub>
pH	7.1 - 7.7	7.0 - 7.4	7.0 - 7.4	7.0 - 7.4	7.1 - 7.3
Detergents	optional	non-ionic detergent optional; 0.5 - 1.0 mL/L Triton X-100 recommended	in 1 L: "1 mL (...) or Triton X-100 (...) have been found to be satisfactory"	non-ionic detergent optional; 0.5 - 1.0 mL/L Triton X-100 recommended	1 mL/L detergent
Time (duration of conversion)	typically 15 min to 90 min; in presence of COHb ≥ 60 min	5 min to 30 min (pure COHb)	3 to 4 min	5 min to 30 min (pure COHb)	≥ 90 min (max. 24 h)
Separation	Centrifugation	Filtration	NA	Filtration	Centrifugation
ε <sub>540</sub>	11.0 m <sup>2</sup> min <sup>-1</sup>	11.0 m <sup>2</sup> min <sup>-1</sup>	11.0 m <sup>2</sup> min <sup>-1</sup>	11.0 m <sup>2</sup> min <sup>-1</sup>	11.0 m <sup>2</sup> min <sup>-1</sup>
T	18 - 24 °C	18 - 25 °C	20 - 25 °C	20 - 25 °C	20 - 24 °C

Total Hb –  
implementation of a reference system

# Reference System

(Panteghini, Clin Biochem Rev. 2007;28;97, Traceability, Reference Systems and Result Comparability)

- Reference measurement procedure (RMP) ✓
- Reference material not required
- Reference laboratories ✓
- Collaborating in a network
  - PTB
  - Calibration Laboratory A
  - Calibration Laboratory B
  - Calibration Laboratory C

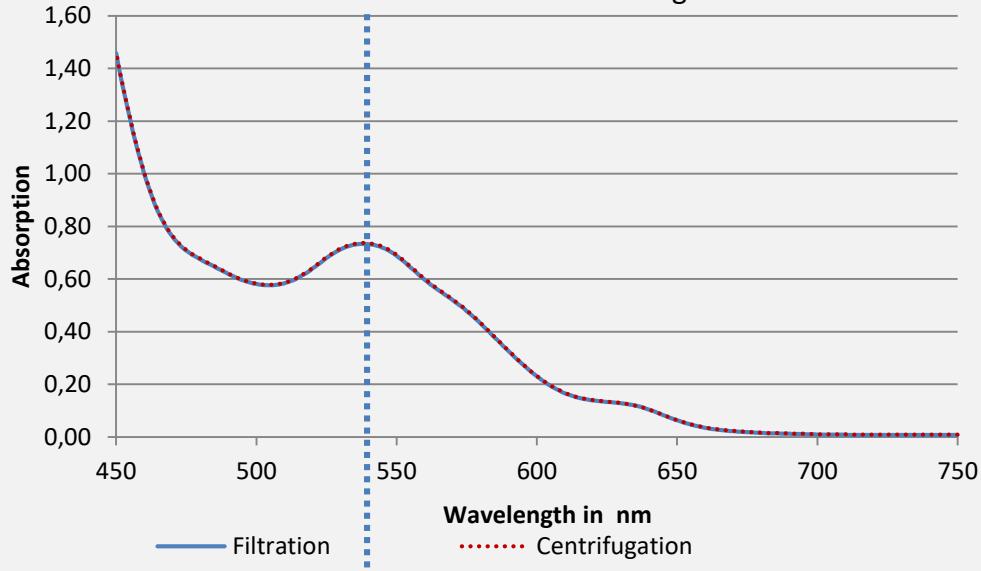
NMI  
accredited according to  
ISO 15195 &  
ISO 17025

# RMP

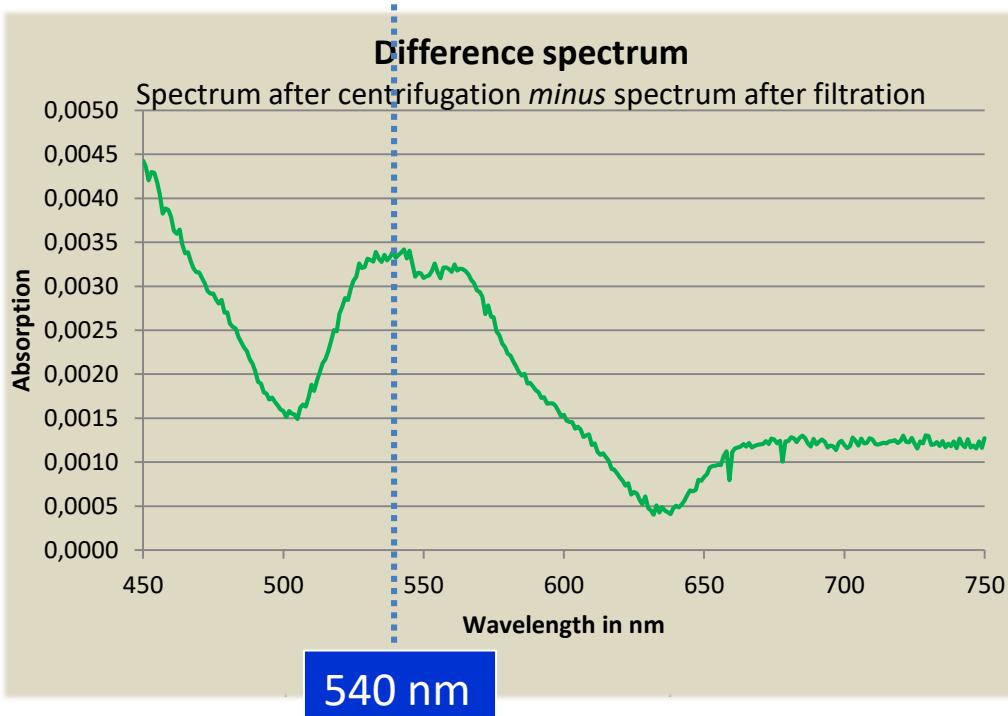
- Influence of measurement parameters  
→ investigated
- Optimization  
→ e.g. narrowed tolerance limits  
→ preferably in line with standards
- Expanded measurement uncertainty:  
→  $U_{(\text{BMC})} = 1.1\% \ (k = 2)$

Cer

### Spectrum of Hb reaction mixture after filtration and after centrifugation



### Difference spectrum



# Evaluation

## A) Comparison

- Samples:  $n = 24$
- Source: EQA organizations



- Period: 1 year
- Sites: 4 laboratories

EQA sample	PTB (g/L)		Laboratory A (g/L)		Laboratory B (g/L)		Laboratory C (g/L)		Difference	Difference	Difference
	Hb conc	$u_{NMI}$	Hb conc	$u_{cA}$	Hb conc	$u_{cB}$	Hb conc	$u_{cC}$	A-B	A-C	B-C
1	88.8	0.7	88.8	0.5	89.3	0.6			not sig.		
2	60.8	0.5	60.6	0.3	60.8	0.4			not sig.		
3			134.6	0.7	134.5	0.8	134.1	0.8	not sig.	not sig.	not sig.
4			159.7	0.9	159.7	1.0	156.6	1.0	not sig.	significant	significant
5			138.4	0.8	137.2	0.8	137.7	0.8	not sig.	not sig.	not sig.
6			65.4	0.4	65.8	0.4	66.3	0.4	not sig.	not sig.	not sig.
7			88.5	0.5	89.6	0.6	88.9	0.6	not sig.	not sig.	not sig.
8			136.1	0.7	137.0	0.9	137.0	0.9	not sig.	not sig.	not sig.
9			138.6	0.8	138.5	0.9	136.9	0.9	not sig.	not sig.	not sig.
10			65.2	0.4	65.9	0.4	65.7	0.4	not sig.	not sig.	not sig.
11			157.5	0.9	157.8	1.0	155.9	0.9	not sig.	not sig.	not sig.
12			150.9	0.8	151.4	0.9	149.7	0.8	not sig.	not sig.	not sig.
13			157.3	0.9	157.9	1.0	157.8	0.9	not sig.	not sig.	not sig.
14			151.5	0.8	151.1	0.9	151.2	0.9	not sig.	not sig.	not sig.
15			137.5	1.0	138.1	0.9	137.8	0.8	not sig.	not sig.	not sig.
16			136.9	0.9	137.6	0.9	137.6	0.8	not sig.	not sig.	not sig.
17			140.3	0.8	139.8	0.9	137.8	0.8	not sig.	significant	not sig.
18			119.7	0.9	119.7	0.7	118.9	0.7	not sig.	not sig.	not sig.
19	65.3	0.5	64.2	0.4	66.0	0.4	65.9	0.3	significant	significant	not sig.
20	104.0	1.0	102.4	0.6	103.7	0.6	103.7	0.6	not sig.	not sig.	not sig.
21			137.1	0.8	137.6	0.8	137.7	0.8	not sig.	not sig.	not sig.
22			138.2	0.8	138.9	0.8	139.4	0.8	not sig.	not sig.	not sig.
23			75.1	0.8	76.2	0.4	76.1	0.4	not sig.	not sig.	not sig.
24			153.4	0.8	154.5	0.9	155.0	0.9	not sig.	not sig.	not sig.

# Evaluation

## B) CRM

→ JCCRM 912, ReCCS

(*Reference Material Institute for Clinical Chemistry Standards, Japan*)

- Not listed at JCTLM
- The only material using human Hb
- Certified value according to CLSI RMP

CRM level	JCCRM (g/L)		PTB (g/L)		Laboratory A (g/L)		Laboratory B (g/L)		Difference
	Hb conc	$u$	Hb conc	$u_{NMI}$	Hb conc	$u_{cA}$	Hb conc	$u_{cB}$	
912-2 L	78.0	0.5			78.8	0.4	77.1	0.5	significant
912-2 M	137.2	0.8	135.6 <sup>a</sup>	0.45	136.3	0.7	136.1	0.8	not sig.
912-2 H	179.5	1.1			177.3	1.0	178.7	1.1	not sig.

<sup>a</sup> from Frank et al., Anal Bioanal Chem 2017;409:2341-51.

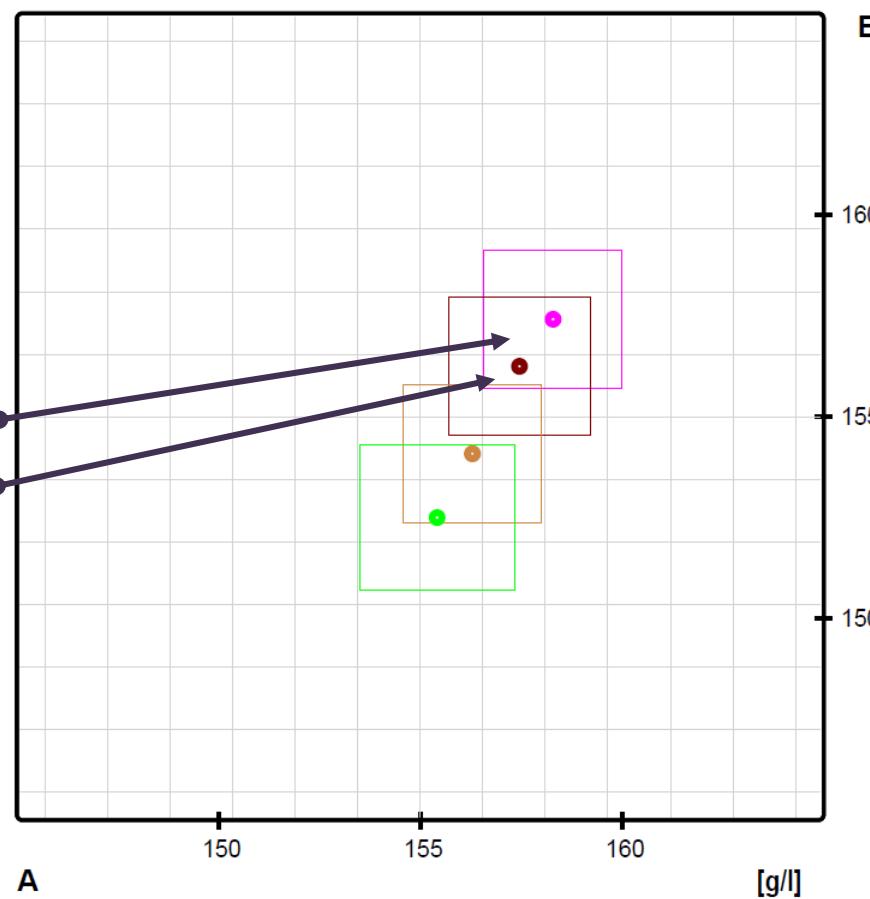
# Evaluation

## C) RELA EQA for reference laboratories

- Addition of measurand to the programme
- 4 participants (2018)

Laboratory	Sample A	exp. MU	Sample B	exp. MU
1	158.3	1.7	157.4	1.7
2	157.4	1.7	156.3	1.7
3	156.3	1.7	154.1	1.7
4	155.4	1.9	152.5	1.8

Total Hemoglobin



EQA sample	Mean of participants (g/L)	Δ to Lab A (%)	Δ to Lab B (%)	Δ to Lab C (%)
<b>1</b>	<b>Mean (%)</b>	99.7	99.2	99.5
<b>2</b>	Max (%)	101.6	100.4	101.5
<b>3</b>	Min (%)	98.4	97.2	97.4
<b>4</b>	SD (%)	0.7	0.7	1.1
<b>6</b>	65	99	99	98
<b>7</b>	89.9	102	100	101
<b>8</b>	136	100	99	99
<b>9</b>	137	99	99	100
<b>10</b>	65	100	99	99
<b>11</b>	158	100	100	101
<b>12</b>	150	99	99	100
<b>13</b>	157	100	99	100
<b>14</b>	150	99	99	99
<b>15</b>	138	100	100	100
<b>16</b>	137	100	100	100
<b>17</b>	138	98	99	100
<b>18</b>	119	99	99	100
<b>19</b>	64.2	100	97	97
<b>20</b>	103	101	99	99
<b>21</b>	136	99	99	99
<b>22</b>	137	99	99	98
<b>23</b>	74.5	99	98	98
<b>24</b>	153	100	99	99

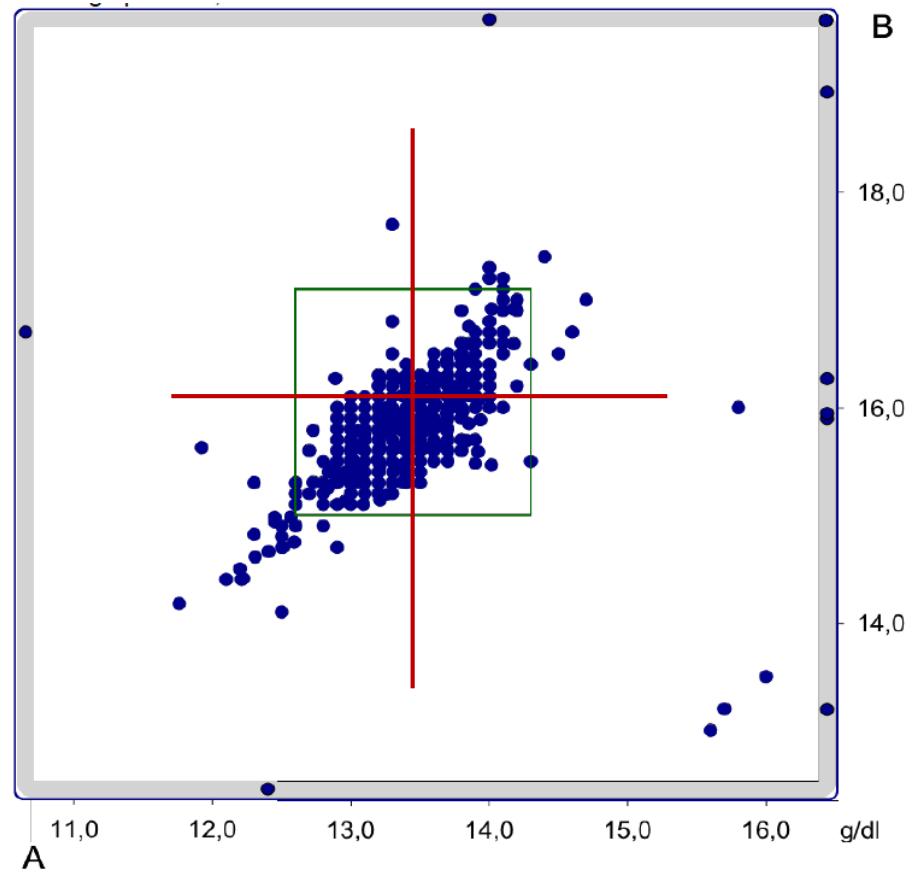
Total Hb –  
implementation of a reference system

Crosses are  
draft sketches

# Routine Labs EQA

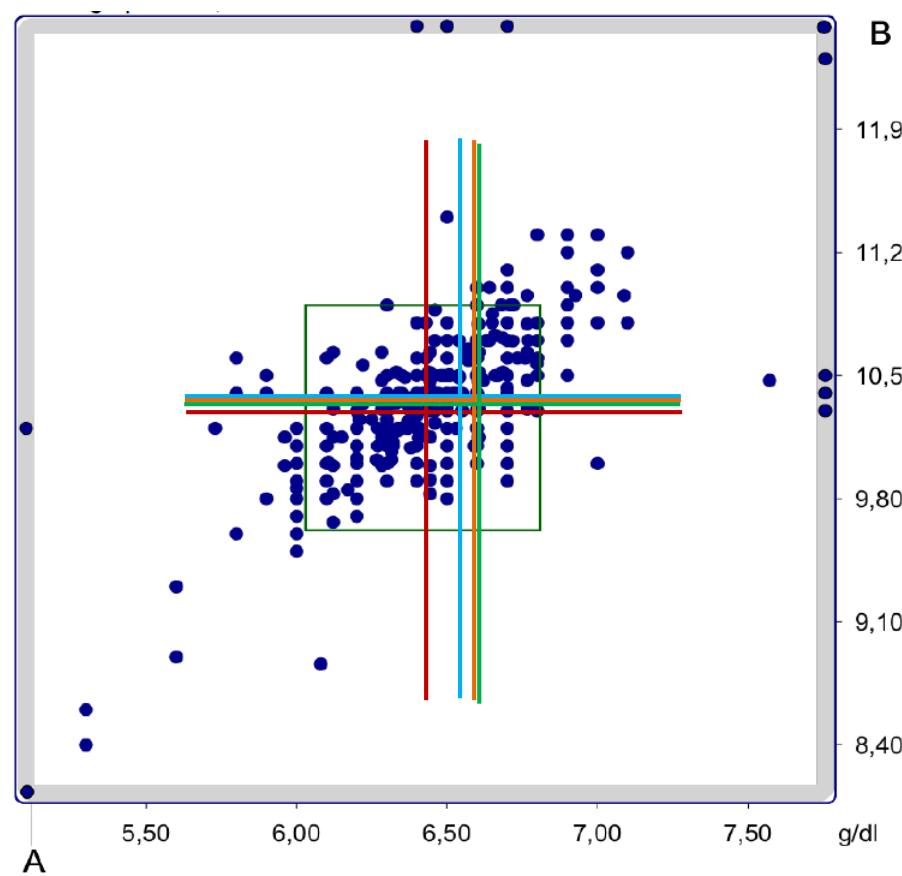
RfB: HA 3/19,  $n = 1585$  participants

Sample	A (g/dL)	B (g/dL)
RMV (1):	13.4	16.1
Mean:	13.4	15.9
Median:	13.4	15.9



RfB: HA 4/17,  $n = 1577$  participants

Sample	A (g/dL)	B (g/dL)
RMVs (4):	6.42–6.60	10.24–10.40
Mean:	6.42	10.3
Median:	6.40	10.3



# Routine Labs EQA

- Reference method
  - in line with  
Routine laboratories
  - in line with  
common reference intervals

# Reference measurement service – „implementation“

- 3 Calibration laboratories
  - officially accredited (ISO 15195 and ISO 17025)
- NMI involved (*PTB, Physikalisch-Technische Bundesanstalt*)
  - RELA participation desired
- EQA target values
  - RfB: calibration lab
  - Instand: calibration lab

! EQA target values of Hb must be RMV of accredited CL's in Germany !

# Conclusion

- HiCN principle accepted as a reference
- HiCN settings differ between standards
- Optimized Reference Method
- Evaluation
  - Accredited calibration laboratories (3) and NMI (PTB)
  - Comparison study: good agreement
  - In line with common reference intervals/routine labs
  - Reference measurement service offered
- Manuscript submitted (*cCLM*, 11-2019)
- Objective: JCTLM listing of RMP / of services