Stability of haemiglobincyanide standards

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Introduction

This journal published the first recommendations and requirements for haemoglobinometry in human blood by the International Committee for Standardization in Haematology of the European Society of Haematology in 1965.¹ The recommendations were discussed further and adopted at the world level during the XIth International Congress of Haematology in 1966² and have since been reviewed and modified on the basis of continued experimental studies^{3,4}; the latest revision can be found on p 271.

The recommended reference method for the determination of the haemoglobin concentration in blood is spectrophotometric measurement after conversion of haemoglobin to haemiglobincyanide (HiCN) (cyanmethemoglobin, CNmetHb). The measuring instrument should be checked as to absorbance with HiCN standards. The Dutch National Institute for Public Health (and Environmental Protection) (RIV (M)) prepared HiCN solutions for distribution as a reference standard for haemoglobinometry in 1961 and offered such a solution to ICSH as candidate international HiCN reference preparation in 1963. ICSH issued the first international HiCN reference solution in 1966 and the World Health Organisation (WHO) established such solutions as the international HiCN reference preparation in 1968.⁵ RIV has continued to produce the international HiCN reference preparations, now international HiCN standards, through 1987 and the WHO has accepted subsequent preparations as the second, third, etc., international standard. New standards were originally produced every year, checked for nine to 12 months, then issued for a period of two years. This ensured that, should a solution suddenly deteriorate, a second standard would be available for distribution. International HiCN standards are used to assign values to secondary HiCN reference solutions, and as a reference against which to judge the purity of such HiCN solutions.

As stability data on these standards, stored at 4°C, in the dark, accumulated,⁶⁻⁹ it was decided in 1979 to go to a three year production cycle and to issue the standards with a six year expiration date. This report summarises measurement data of international HiCN standards stored at 4°C, in the dark, for up to 27 years.

Methods

International HiCN standards are aqueous solutions of HiCN prepared from washed human erythrocytes with an HiCN concentration of 550–600 mg/l.¹⁰ To ensure sterility, the solution

is filtered, under pressure, through a membrane filter with a mean pore diameter of 0.20–0.45 µm and, under aseptic conditions, dispensed in 10 ml ampoules of brown borosilicate glass.¹¹ The batch prepared in 1987 (lot 70 600; table 1) was dispensed in ampoules of clear borosilicate glass. The content and purity of the standards are determined and checked by ICSH appointed reference laboratories in Europe, Japan, and the USA at three month intervals before issue nine to 12 months after preparation. The reference laboratories continue checking at six monthly intervals until the assigned expiration date. The content is calculated from the measurements at 540 nm and is based on a value of 11.0 l.mmol⁻¹.cm⁻¹ for the absorptivity of HiCN at that wavelength.^{12,13} The purity is checked by measuring at 750 nm ($A_{\text{HiCN}}^{750} \leq 0.003$ per cm light-path) for absence of turbidity, by determining the quotient of the absorbances at 540 and 504 nm ($1.59 \leq A_{\text{HiCN}}^{540}/A_{\text{HiCN}}^{504} \leq 1.63$) and by visual inspection of the absorbance curve (fig 1) from 750 down to 450 nm².

All batches of international HiCN standard issued have been stored at 4°C in one of our laboratories and have been checked regularly. Spectrophotometric measurements have been performed with a Beckman DU, half-intensity bandwidth 0.52 and 0.42 nm at 540 and 504 nm respectively, and/or an Optica CF4 grating instrument, total bandwidth 0.5, 0.13,

Figure 1 Absorption curve of international HiCN standard batch 70 500. Varian Cary 2290 spectrophotometer, 1.000 cm lightpath, water as blank. For pure HiCN solutions, $A_{\text{HiCN}}^{750} \leq 0.003$ and $1.59 \leq A_{\text{HiCN}}^{540}/A_{\text{HiCN}}^{504} \leq 1.63$.

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Table 1 Long term stability of international HiCN standards stored at 4°C

Batch number (year prepared)																		
40400 (1964)		60400 (1966)		70400 (1967)		80400 (1968)		90400 (1969)		00400 (1970)		10400 (1971)		20400 (1972)		30400 (1973)		
Absorbance	$A^{540} \dagger$	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	
	A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}	
Age of batch in years																		
0	0.391	1.61	0.386	1.61	0.405	1.62	0.383	1.60	0.389	1.61	0.395	1.61	0.413	1.61	0.408	1.61	0.403	1.61
0.25			0.387	1.61			0.381	1.60	0.389	1.61	0.395	1.61	0.414	1.60	0.408	1.61	0.402	1.61
0.5			0.387	1.61	0.404	1.62	0.386	1.61 ⁵	0.388	1.62	0.396	1.61	0.415	1.61	0.408	1.61	0.403	1.62
0.75			0.387	1.61			0.383	1.61										
1	0.389	1.61	0.388	1.61	0.405	1.59 ⁵			0.388	1.61	0.400	1.62			0.408	1.62	0.402	1.61
2-5	0.391	1.61	0.387	1.61	0.404	1.62	0.386	1.61	0.390	1.61	0.393	1.60	0.413	1.61	0.404	1.61	0.401	1.61
3-3.5	0.391	1.62	0.388	1.61	0.404	1.61	0.386	1.61	0.387	1.61	0.393	1.61	0.412	1.61	0.407	1.60	0.403 ⁵	1.60 ⁵
5-5.5	0.393	1.61	0.386	1.61	0.404	1.62	0.384	1.61	0.387	1.60	0.393	1.60	0.411	1.61	0.402*	1.60		
9-10	0.390	1.60	0.386	1.61	0.404	1.61	0.381	1.60			0.389	1.59	0.405	1.61				
13-15			0.381	1.60	0.405	1.58 ⁵	0.386 ⁵	1.60 ⁵	0.384	1.59	0.391	1.58	0.405	1.60 ⁵			0.400	1.60 ⁵
16-17	0.387 ⁵	1.59	0.380	1.60	0.401	1.59 ⁵	0.382	1.59	0.389	1.59	0.386	1.50 ⁵					0.396 ⁵	1.58
18-19	0.391 ⁵	1.58	0.380 ⁵	1.59 ⁵	0.399 ⁵	1.60	0.380 ⁵	1.60 ⁵					0.403	1.59 ⁵				
20-21	0.382	1.56 ⁵					0.379 ⁵	1.59 ⁵	0.383*	1.60	0.386	1.57					0.393 ⁵	1.53 ⁵
22													0.397 ⁵	1.53 ⁵				
23	0.380*	1.57	0.380	1.58	0.398 ⁵	1.59					0.384*	1.56						
25							0.378	1.58										
26					0.401 ⁵	1.60												
27			0.372	1.62														
Batch number (year prepared)																		
40500 (1974)		50500 (1975)		60500 (1976)		70500 (1977)		80500 (1978)		90500 (1979)		20600 (1982)		40600 (1984)		70600 (1987)		
Absorbance	$A^{540} \dagger$	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	A^{540}	
	A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}		A^{540}	
Age of batch in years																		
0	0.382 ⁵	1.60	0.379	1.61	0.392 ⁵	1.60	0.394 ⁵	1.60	0.396	1.60	0.392 ⁵	1.61	0.382 ⁵	1.60	0.399 ⁵	1.60	0.404	1.61
0.25	0.380	1.61	0.381	1.61	0.394	1.61	0.396	1.61	0.393	1.61	0.392	1.61	0.380 ⁵	1.61			0.404	1.61
0.5	0.381	1.60	0.381 ⁵	1.61	0.392	1.61	0.395	1.61	0.394	1.60	0.393 ⁵	1.61			0.402	1.60 ⁵	0.400	1.59
0.75											0.392 ⁵	1.61			0.402	1.60 ⁵		
1	0.378	1.61	0.379 ⁵	1.60	0.393 ⁵	1.60	0.395	1.61	0.393 ⁵	1.61	0.392 ⁵	1.60	0.381 ⁵	1.59	0.401	1.61	0.403	1.61
2-2.5			0.380	1.60	0.391	1.61	0.392	1.61	0.393	1.61	0.391 ⁵	1.60	0.378	1.60	0.396 ⁵	1.60 ⁵	0.402	1.61
3-4					0.391	1.62			0.392 ⁵	1.60 ⁵	0.390 ⁵	1.60	0.379 ⁵	1.59	0.398	1.60	0.402	1.60
5-6			0.379 ⁵	1.61	0.387 ⁵	1.61 ⁵	0.391 ⁵	1.61	0.393 ⁵	1.60	0.386 ⁵	1.59	0.377	1.60	0.399	1.59 ⁵	0.401 ⁵	1.60
8-11	0.376	1.61	0.377 ⁵	1.61	0.393	1.61	0.392 ⁵	1.61	0.395 ⁵	1.60 ⁵	0.386	1.59 ⁵	0.376	1.58	0.397 ⁵	1.59 ⁵		
11.5-13	0.377 ⁵	1.61	0.379	1.60 ⁵			0.392	1.59 ⁵	0.390 ⁵	1.60			0.374 ⁵	1.59 ⁵				
13.5-15			0.376	1.59 ⁵	0.393	1.58			0.395 ⁵	1.59	0.394	1.59						
16	0.376 ⁵	1.59 ⁵			0.389 ⁵	1.59	0.396 ⁵	1.60 ⁵										
17																		
18			0.380 ⁵	1.59 ⁵														
19	0.370	1.63																

* Last ampoule in storage; † absorbance values are the mean of duplicate or quadruplicate measurements. A “fourth” decimal place is indicated by a superscript numeral—for example, 0.387⁵.

and 0.13 nm at 750, 540, and 504 nm, respectively, and/or a Hewlett-Packard 8450A reversed optics, diode array instrument, total bandwidth 2 nm at all wavelengths of measurement, in a layer thickness of 1.000 cm, against water as blank. The spectrophotometers were checked regularly as to wavelength by means of the mercury emission lines 546.1 and 491.6 nm; the absorbance was checked on each day of measurement by means of a Corning HT yellow glass filter⁷ certified by the US National Bureau of Standards (now National Institute of Standards and Technology). The absence of stray light was verified upon installation of the instruments by means of glass or liquid cut off filters.¹⁴

Results and Discussion

Table 1 summarises the measurement results at 540 nm and the calculated quotients A^{540}/A^{504} of HiCN standards issued since 1966. The first, 40 400, was a pilot preparation and was not issued for distribution. Each value is the mean of two or more measurements by one or two operators, measuring a single vial of standard on one to three instruments. Occasionally, a second ampoule was measured for verification. Measurement results at 750 nm

have not been included because values of 0.002 to 0.003 have consistently been found as long as A^{540}/A^{504} remained ≥ 1.59 ; as A^{540}/A^{504} was found to decrease, A^{750} increased. For example, A^{540}/A^{504} of standards 10 400 and 30 400 were found to be 1.53 after 22 and 21 years respectively; a value of 0.006 was measured for A^{750} in both cases.

We conclude from these studies that properly prepared, sterile, international HiCN standards are generally stable—that is, a decrease in A^{540}_{HiCN} no more than 2% over about 15 years. The standards may deteriorate progressively after 20 years of storage at 4°C. The cause and nature of this deterioration are not known. Although some data on the stability of HiCN standards stored at room temperature or higher have been published, no long term, controlled studies have been reported. Holtz¹¹ quotes a loss in absorbance at 540 nm of about 1% per year for standards stored at 20°C and about 8% per year when stored at 37°C; van Assendelft *et al.*⁶ showed that HiCN standards stored at room temperature (18–24°C) generally deteriorate after about 1.5 years.

In recent preliminary accelerated degradation pilot studies variable results were obtained with eight HiCN solutions stored at 20, 37, 45, and 50°C for eight weeks and one

solution stored for six months. For solutions stored at 20°C, no decrease in A^{540} was found after six months; A^{540} of solutions stored at 37°C decreased from 0.2 to 0.5% per month; at 45°C the decrease ranged from 0.05 to 1% per week and at 50°C from 0.15 to 3.5% per week. Further controlled studies are required to quantify and characterise the nature of the degradation of HiCN solutions when stored at temperatures above 20°C.

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