az sint-jan
brugge - oostende av

Critically Appraised Topic

Belang en bepaling van hematocriet in stamcelafereseproducten

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Inleiding: hematopoietische stamceltransplantatie

Indicaties

AZ Sint-Jan

- 2e grootste centrum van Vlaanderen
- Jaarlijks 70 Tx, 110 collecties

Autoloog vs. allogeen

- Weefselincompatibiliteit: HLA vs ABO

HSC-types (navelstrengbloed, beenmerg, aferese perifeer bloed)

Probleemstelling

Lage hematocrietwaarden

Deel 1
Evidentie en richtlijnen

Deel 2
Evaluatie automatische celtellers

Deel 1: Evidentie en richtlijnen

ABO-incompatibiliteit

Majeure incompatibiliteit

Mineure incompatibiliteit

Bidirectionele incompatibiliteit

		Ontvanger			
	Bloedgroep	A	B	AB	O
Donor	A		M en m	m	M
	B	M en m		m	M
	AB	M	M		M
	O	m	m	m	

Table 2 Immunohematological consequences of ABO-incompatible transplantation

Incompatibility	*Consequence*	*Cause*
ABO major	Acute hemolysis	Infusion of incompatible red cells
	Delayed granulocyte and platelet engraftments	Loss of HPC from processing to remove red cells. Expression of ABO antigens on granulocytes and platelets
	Delayed red cell engraftment	Host anti-donor isoagglutinins
	Pure red cell aplasia	Persistence of anti-donor isoagglutinins
ABO minor	Acute hemolysis	Donor plasma with high isoagglutinin titers
	Delayed hemolytic reaction	Passenger lymphocytes producing anti-host isoagglutinins

Abbreviation: HPC = hematopoietic progenitor cell.

Rowley et al. 2011

Aanpak majeure ABO-incompatibiliteit

RBC-depletie

Echter mogelijk verlies van stamcellen!

Hydroxyethylzetmeel, dextraan

Ficoll-Paquegelgradiënt

Centrifugatie

Manueel/semi-automatisch

Reductie isoagglutininetiters

Plasma-uitwisseling

Immunoadsorptie (*ex* en *in vivo*)

Gefractioneerde toediening

Aanpak majeure ABO-incompatibiliteit

Internationale richtlijnen

FACT-JACIE international standards, 2015

B6.4.3 Allogeneic donors and allogeneic recipients shall be tested for ABO group and Rh type using two independently collected samples. Discrepancies shall be resolved and documented prior to issue of the cellular therapy product.

B6.4.4 A red cell antibody screen shall be performed on allogeneic recipients.

B7.6.2 There shall be a policy for volume of ABO-incompatible red cells in allogeneic cellular therapy products.

D5.1.4 Processing of ABO-incompatible cellular therapy products to include a description of the indication for and processing methods to be used for red cell and plasma depletion.

Aanpak majeure ABO-incompatibiliteit

Belgische richtlijnen

Marrow Donor Program Belgium (MDPB), 2015

Marrow Donor Program Belgium - Registry
Motstraat 40 2800 Mechelen
Tel: (32) - 15 44 33 96 Fax: (32) - 15 44 36 56
Email : MDPB-registry@rodekruis.be

4.2.5 The Donor Center, when requested for blood from the donor for Verification Typing (Confirmatory Typing) shall perform the following tests:

infectious disease markers (IDM)	
Syphilis	
HBs Ag	
HBcore antibody	
Anti-HIV1-2 antibodies	
Anti-CMV antibodies	
Anti-hepatitis C virus (anti-HCV) antibodies	
Other tests	
ABO and Rh typing	if not already performed

MARROW DONOR PROGRAM BELGIUM

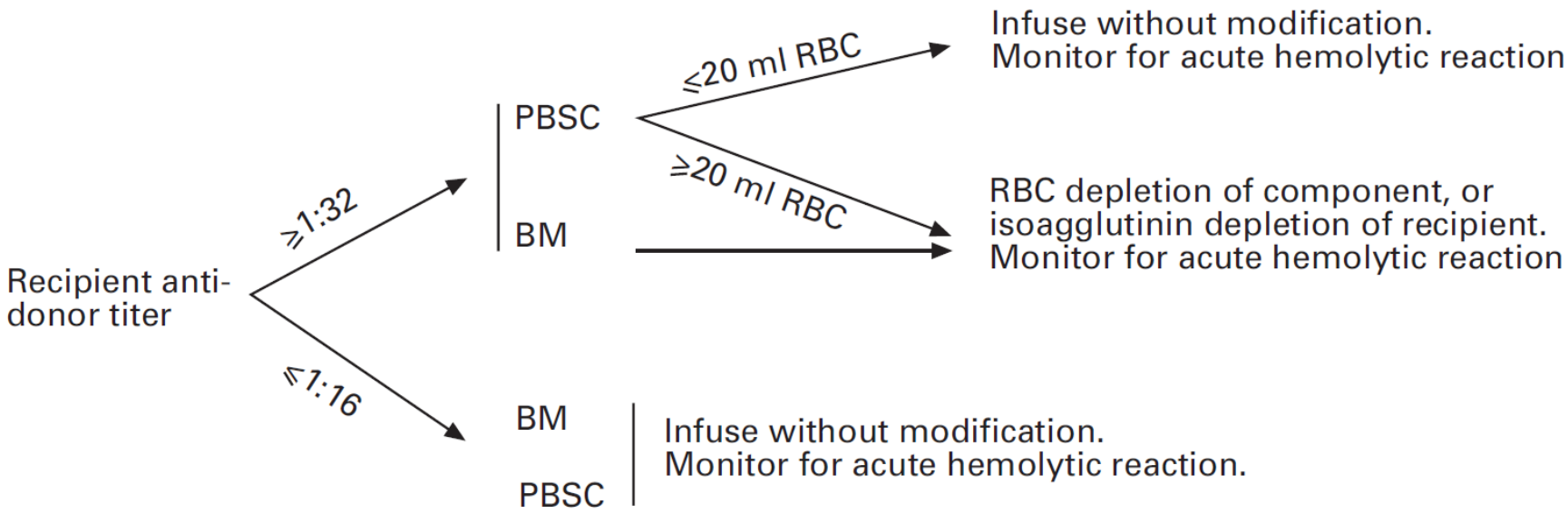
STANDARDS DONOR

4.7.1 Samples to be obtained at time of first collection

Blood for ABO and Rh type: a test for ABO group and Rh type shall be performed on blood obtained from the donor at the time of the first collection (or on the first product collected).

4.7.6.3 Additional processing, such as the removal of T cells, incompatible red cells, or plasma, should be performed by the Hematopoietic Stem Cell Bank associated with the Transplant Center or in another Hematopoietic Stem Cell Bank designated by the Transplant Center.

Aanpak majeure ABO-incompatibiliteit



Rowley et al. 2001

Aanpak majeure ABO-incompatibiliteit
Maximaal RBC-gehalte

Janatpour et al. 2008

'Clinical outcomes of ABO-incompatible RBC transfusion'

Volume of ABO-Incompatible RBCs Transfused vs Outcome and Symptoms for 48 Patients

	≤50 mL	>50 mL
No. of patients	12	36
Survived	12	30
Died	0	6
No. of patients without signs or symptoms	9	13
No. of patients with signs or symptoms	3	23
Acute hemolytic transfusion reaction	3	16
Renal failure	0	10
Shock	1	3
Disseminated intravascular coagulopathy	0	3

Aanpak majeure ABO-incompatibiliteit
Maximaal RBC-gehalte

Nederlandse richtlijnen

Centraal BeleidsOrgaan (CBO) Richtlijn bloedtransfusie, 2011

Aanbevelingen 4.2.6

1.	Ter preventie van hemolyse bij toediening van een major ABO-incompatibel stamcel-/beenmerg transplantaat aan een volwassen ontvanger dient bij IgG en/of IgM titer > 16 te worden gestreefd naar < 15 mL erytrocyten in het transplantaat. De toedieningsnelheid moet aangepast worden aan de titer. Bij kinderen is < 10 mL erytrocyten aanbevolen.

Aanpak majeure ABO-incompatibiliteit
Maximaal RBC-gehalte

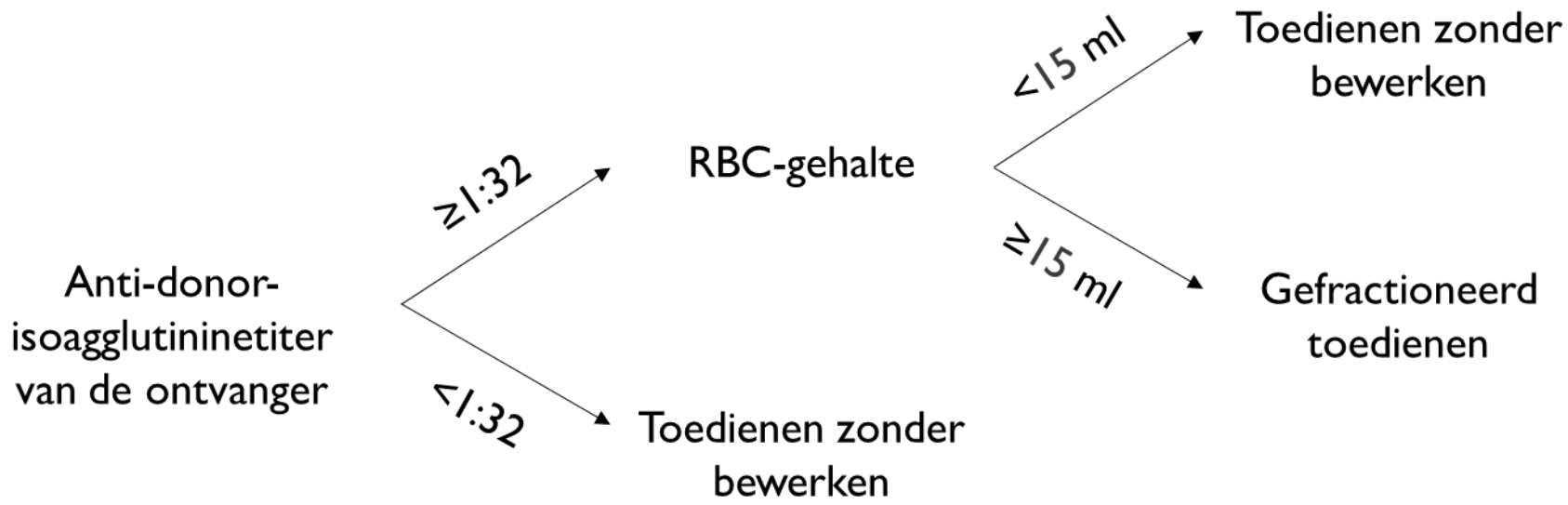
Auteur	Soort publicatie	Aanvaard RBC-gehalte
CBO, 2014	Richtlijnen	< 15ml (volwassene)
		< 10ml (kinderen)
Patrick, 2015	Retrospectieve studie	≤ 3ml/kg (kinderen)
Rowley, 2001	Review	< 20ml
Gajewski, 2006	Review	< 2% HCT (instelling aferesetoestel)
SFGM-TC, 2015	Enquêteresultaten	≤ 0,2ml/kg
ISCT, 2004	Verslag teleconference	≤ 20ml, ≤ 30ml, ≤ 0,15ml/kg, ≤ 0,30ml/kg (volwassene)
		≤ 0,25ml/kg, ≤ 1ml/kg, op advies van clinicus (kinderen)
Braakman, 2011	SOP	< $100*10^9$ RBC per infusie
Griffin	Diavoorstelling	≤ 20ml, ≤ 0,30 ml/kg (kinderen)

Aanpak majeure ABO-incompatibiliteit
Maximale antistoftiter ontvanger

Auteur	Soort publicatie	Aanvaarde antistoftiter (ontvanger)
CBO, 2014	Richtlijnen	IgM en/of IgG anti-B of anti-B < 1:32
Rowley, 2001	Review	<1:32
Witt, 2011	Retrospectieve studie	<1:64
ISCT, 2004	Verslag teleconferentie	<1:16
Daniel-Johnson, 2011	"How to…"	Geen titer

Aanpak majeure ABO-incompatibiliteit
Voorstel beleid AZ Sint-Jan

Collecteren met hematocriet van <2%



+ infusiesnelheid aanpassen aan de antistoftiter
+ monitoring patiënt

Deel 2: Evaluatie celtellers

Evaluatie celtellers

Unicel DxH 800 (Beckman Coulter)

XN-350 (Sysmex)

Evaluatie celtellers

	RBC (*10^6/µL)	MCV (fL)	HCT (%)	WBC (*10^3/µL)	PLT (*10^3/µL)	Differentiatie
Coulter DxH800	✓	✓	✓	✓	✓	✓
Sysmex XN350 impedantie	✓	✓	✓	✓	✓	
Sysmex XN350 optisch	✓				✓	✓
FACSCanto II	✓ (Ref.)			✓ (Ref.)		
Capillaire centrifugatie			✓ (Ref.)			
Manuele microscopie						✓ (Ref.)

	Gemeten parameters		Berekende parameters
DxH 800	**(RBC*MCV)/10**	**=**	**HCT**
XN-350	**(HCT*10)/RBC**	**=**	**MCV**

Evaluatie celtellers

43 aferesestalen
- 24 donoren
- 38 interne, 5 externe
- 13 allogene, 30 autologe transplantaties

Gemiddelde en range
Correlatiecoëfficiënten (R)
Passing-Bablokregressieanalyse: intercept en helling met CI

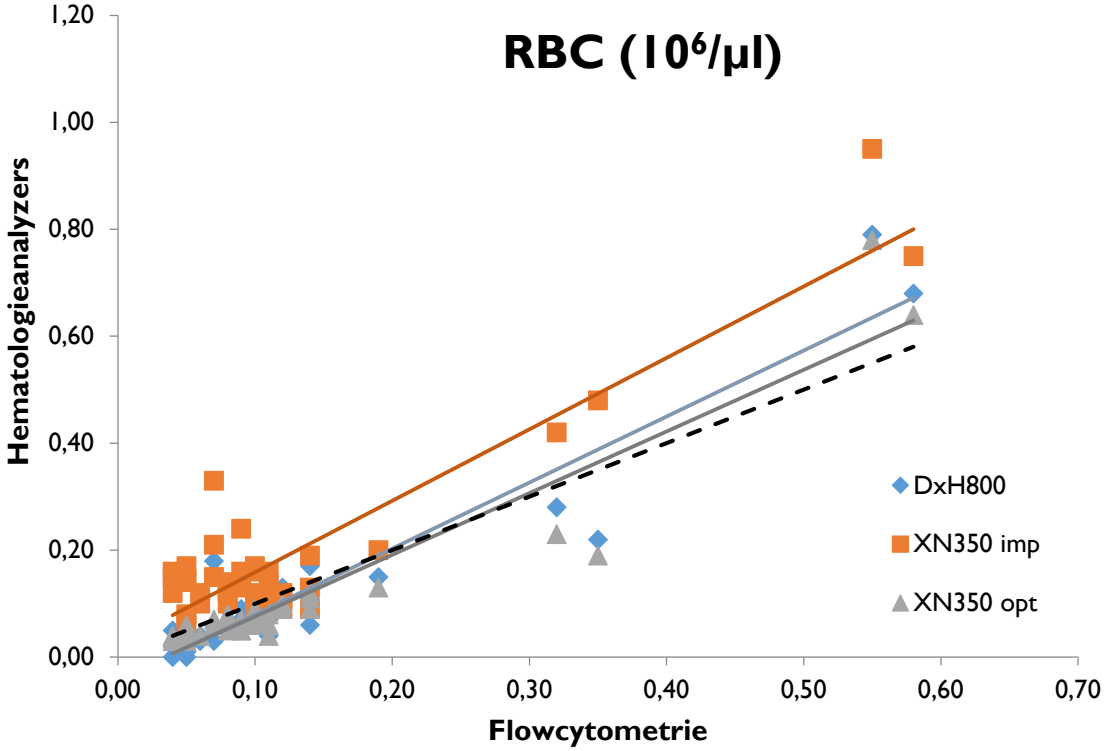
Inter-runprecisie

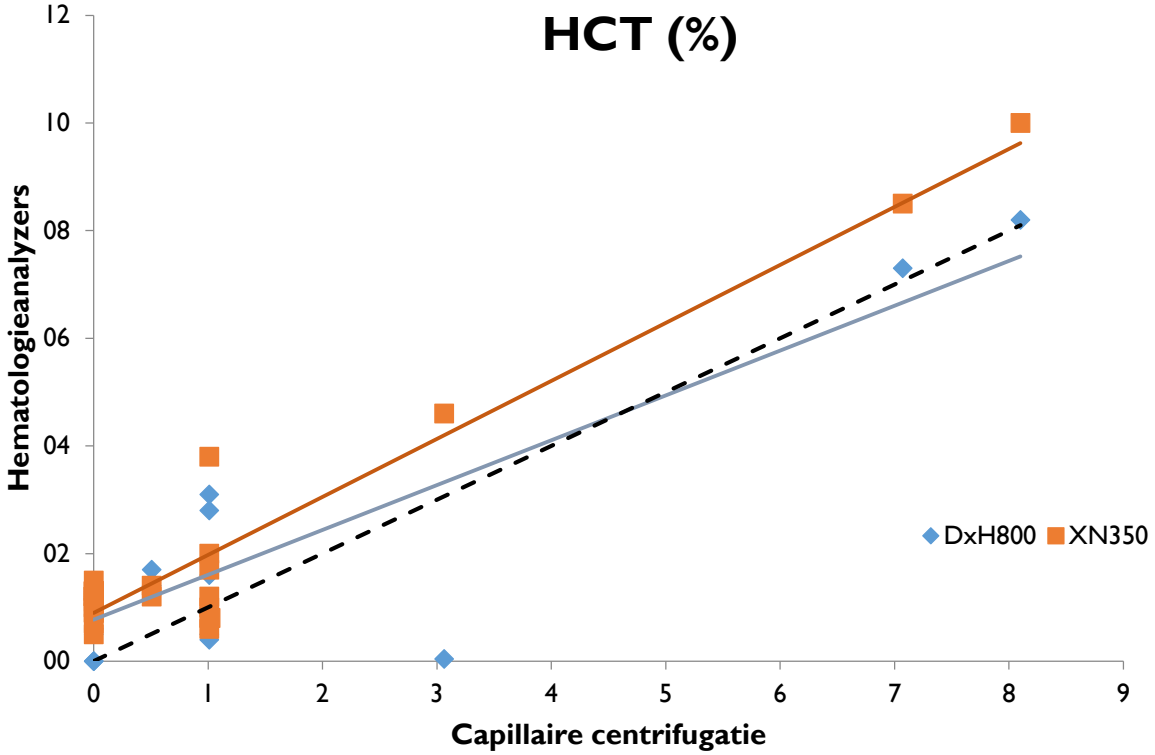
Lineariteit

Evaluatie celtellers
Correlatie

Parameter	Methode			Gemiddelde (range)	R	Intercept (95% CI)	Helling (95% CI)
RBC (*10⁶/µL)	DxH800			0,11 (0,00-0,79)			
	XN350-imp			0,19 (0,06-0,95)			
	XN350-opt			0,10 (0,03-0,78)			
	Ref meth			0,12 (0,04-0,58)			
	DxH800	vs	XN350-imp		0,94	0,06 (0,04-0,08)*	1,13 (0,94-1,56)
	DxH800	vs	XN350-opt		0,98	0,02 (0,01-0,02)*	0,75 (0,60-0,88)*
	XN350-imp	vs	XN350-opt		0,93	-0,01 (-0,04-0,03)	0,47 (0,25-0,78)*
	Ref meth	vs	DxH800		0,95	-0,04 (-0,06--0,03)*	1,15 (1,00-1,38)
	Ref meth	vs	XN350-imp		0,92	0,01 (-0,02-0,05)	1,33 (1,00-1,80)
	Ref meth	vs	XN350-opt		0,94	0,00 (-0,02-0,01)	0,75 (0,63-1,00)
MCV (fL)	DxH800			161,0 (104,1-197,4)			
	XN350			90,1 (60,0-127,3)			
	DxH800	vs	XN350		-0,50	79,4 (13,0-118,4)*	0,05 (-0,17-0,48)*
Hematocriet (%)	DxH800			1,5 (0,0-8,2)			
	XN350			1,7 (0,5-10,0)			
	Ref meth			0,9 (0,0-8,1)			
	DxH800	vs	XN350		0,88	0,12 (-0,10-0,59)	0,98 (0,56-1,20)
	Ref meth	vs	DxH800		0,88	0,78 (0,00-1,10)	1,50 (0,86-3,40)
	Ref meth	vs	XN350		0,96	1,00 (0,90-1,20)*	1,13 (1,00-2,80)

Evaluatie celtellers
Correlatie





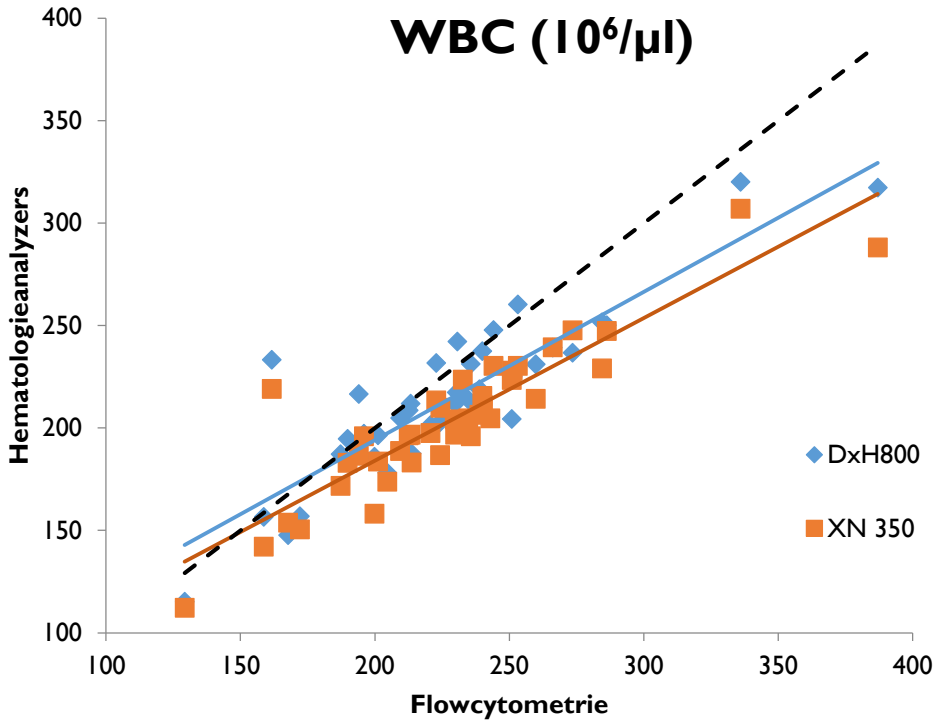
Evaluatie celtellers

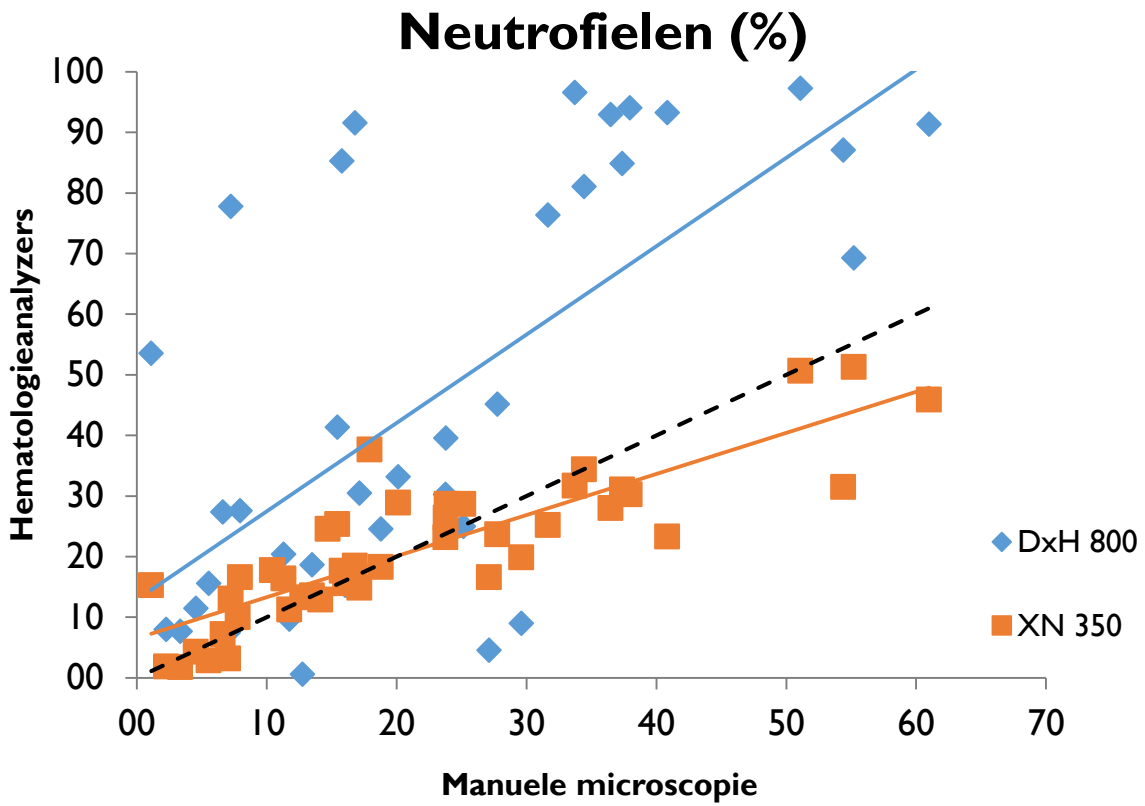
Correlatie

Parameter	Methode			Gemiddelde (range)	R	Intercept (95% CI)	Helling (95% CI)
WBC (*10³/µL)	DxH800			214,5 (115,3-320,2)			
	XN350			203,7 (112,2-307,1)			
	Ref meth			228,2 (129,3-387,0)			
	DxH800	vs	XN350		0,94	-2,3 (-25,9-19,8)	0,95 (0,85-1,07)
	Ref meth	vs	DxH800		0,88	21,4 (-10,7-53,3)	0,83 (0,69-0,97)*
	Ref meth	vs	XN350		0,90	8,5 (-14,0-36,3)	0,86 (0,73-0,96)*
Neutrofielen (%)	DxH800			45,1 (0,6-97,3)			
	XN350			21,3 (1,8-51,3)			
	Ref meth			21,7 (1,1-61,0)			
	DxH800	vs	XN350		0,69	7,6 (3,9-11,3)*	0,33 (0,22-0,45)*
	Ref meth	vs	DxH800		0,68	-9,4 (-31,1-0,3)	2,50 (1,87-3,40)*
	Ref meth	vs	XN350		0,86	2,7 (-0,3-4,7)	0,83 (0,69-1,00)

Evaluatie celtellers

Correlatie





Evaluatie celtellers
Correlatie

Parameter	Methode			Gemiddelde (range)	R	Intercept (95% CI)	Helling (95% CI)
PLT (*10³/µL)	DxH800			1102 (495-2012)			
	XN350-imp			1098 (440-2248)			
	XN350-opt			1245 (530-2367)			
	DxH800	vs	XN350-imp		0,96	-47 (-136-25)	1,03 (0,96-1,12)
	DxH800	vs	XN350-opt		0,98	-69 (-143--1)*	1,21 (1,14-1,29)*
	XN350-imp	vs	XN350-opt		0,96	-15 (-85-20)	1,19 (1,16-1,25)*

Evaluatie celtellers Intra-runprecisie

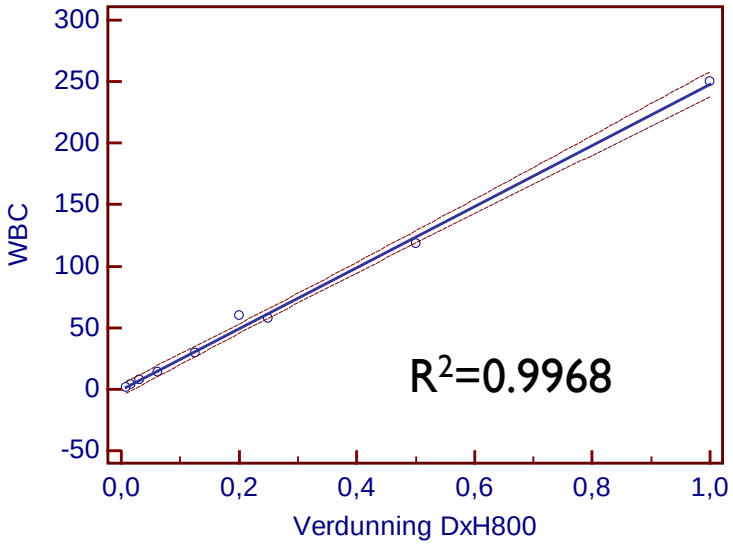
Staal A	DxH 800		XN-350	
n = 10	CV (%)	Gemiddelde	CV (%)	Gemiddelde
RBC	1,99	0,24	1,92	0,49
MCV	0,80	140,0	1,20	85,4
HCT	2,40	3,4	2,84	4,2
WBC	1,93	227,9	0,71	227,8
Neutro%	14,68	79,7	6,38	36,8
PLT	10,16	766	1,14	981

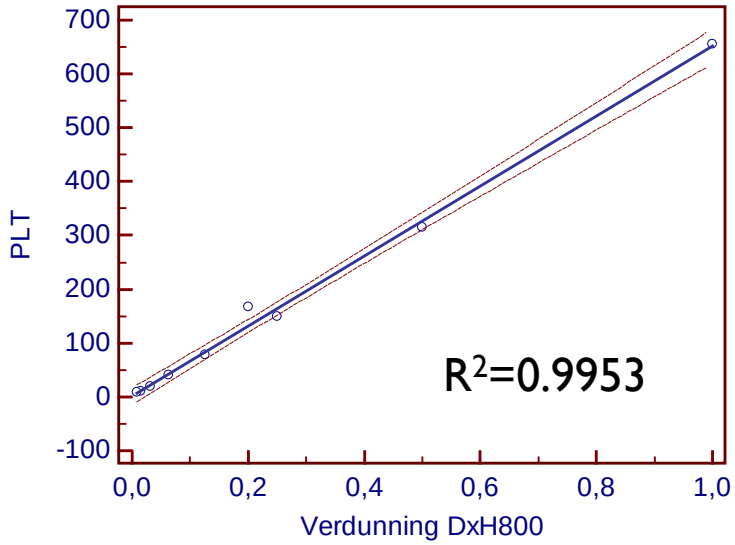
Staal B	DxH 800	
n = 9	CV (%)	Gemiddelde
RBC	3,66	0,09
MCV	1,49	191,6
HCT	4,84	1,7
WBC	2,34	148,1
Neutro%	ND	ND
PLT	3,83	1239

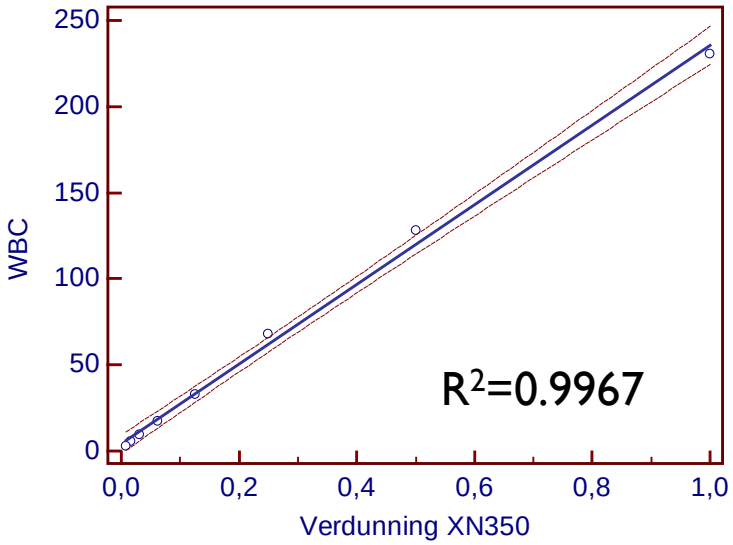
Staal C	XN-350	
n = 9	CV (%)	Gemiddelde
RBC	3,14	0,25
MCV	2,28	66,1
HCT	3,21	1,6
WBC	1,26	209,1
Neutro%	13,81	6,5
PLT	1,35	1468

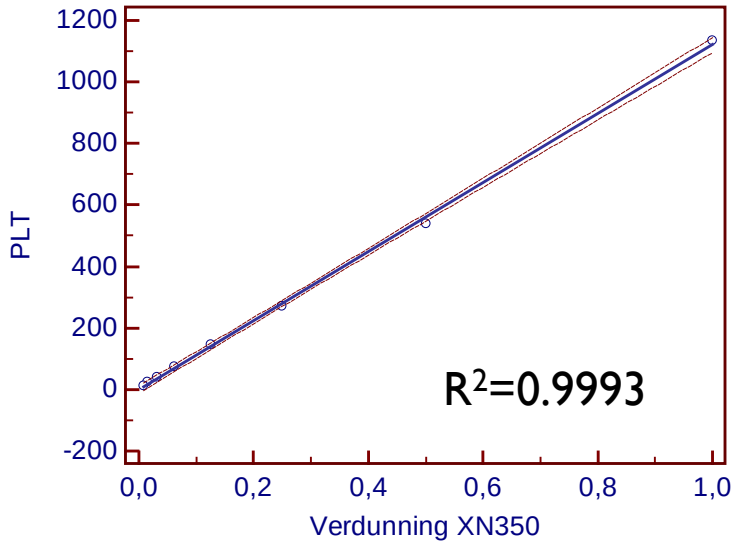
Evaluatie celtellers

Lineariteit



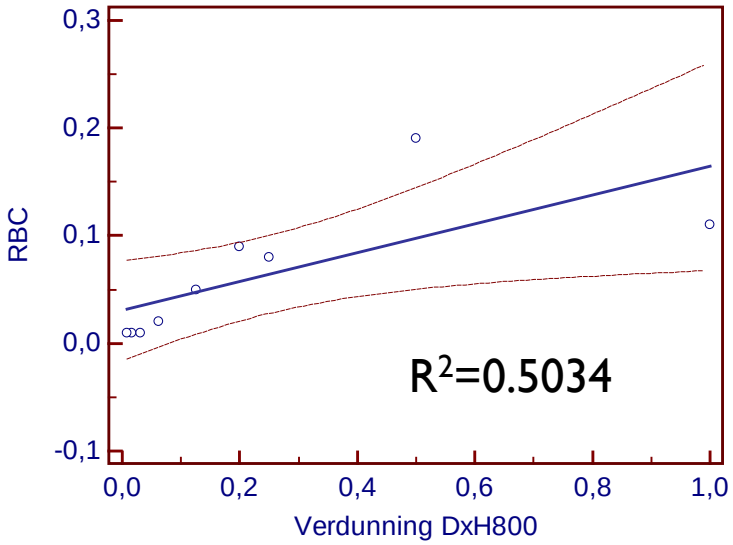


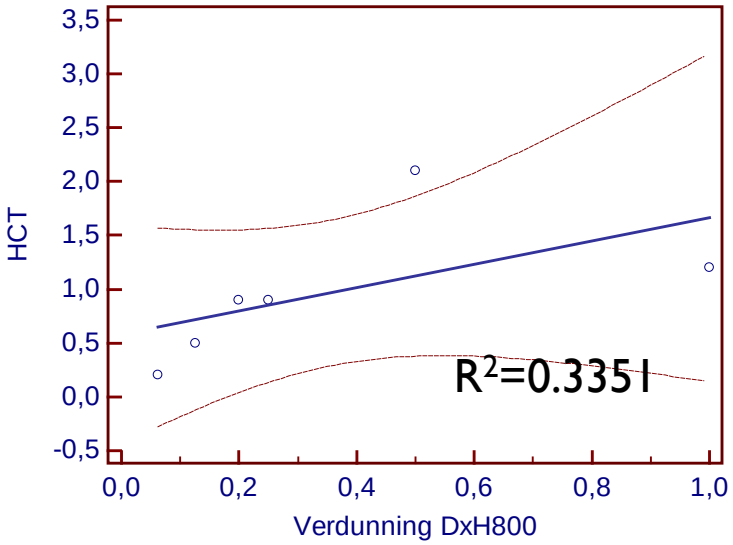


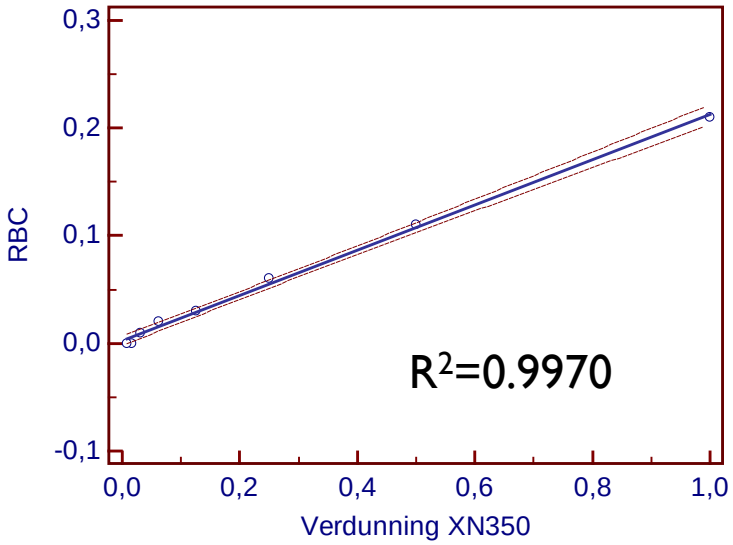


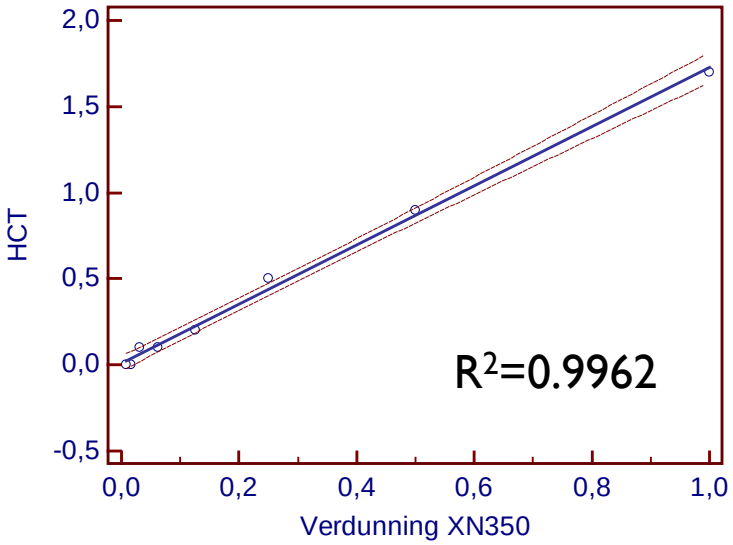
Evaluatie celtellers

Lineariteit



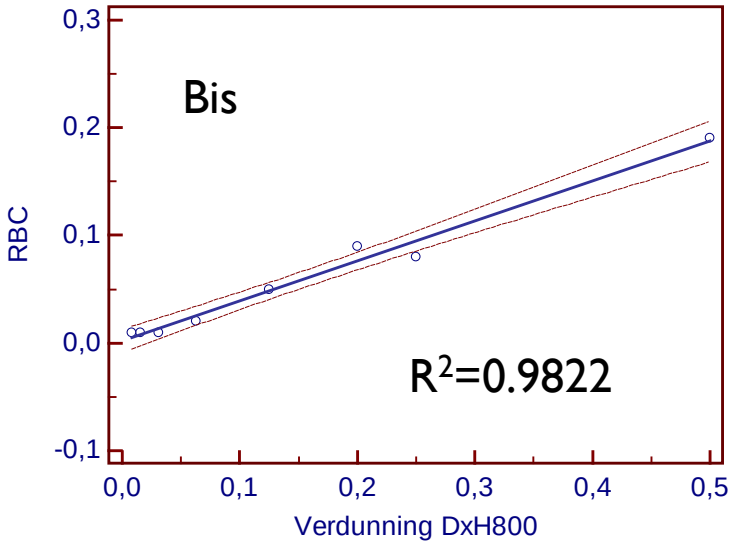


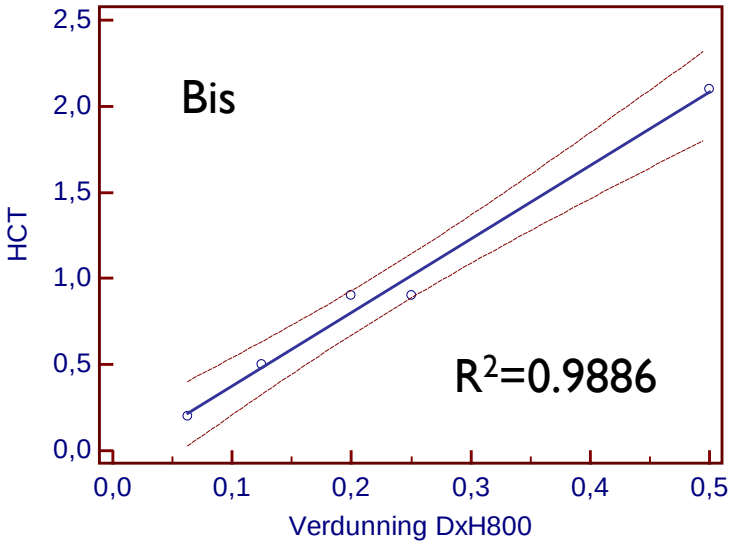


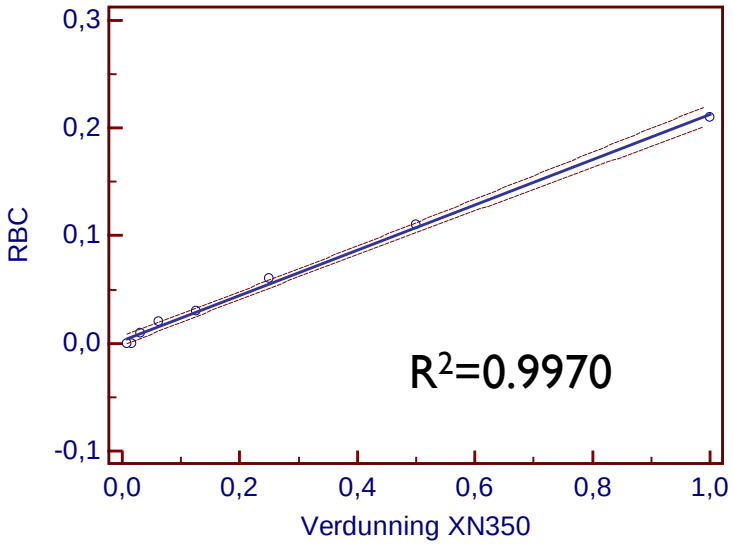


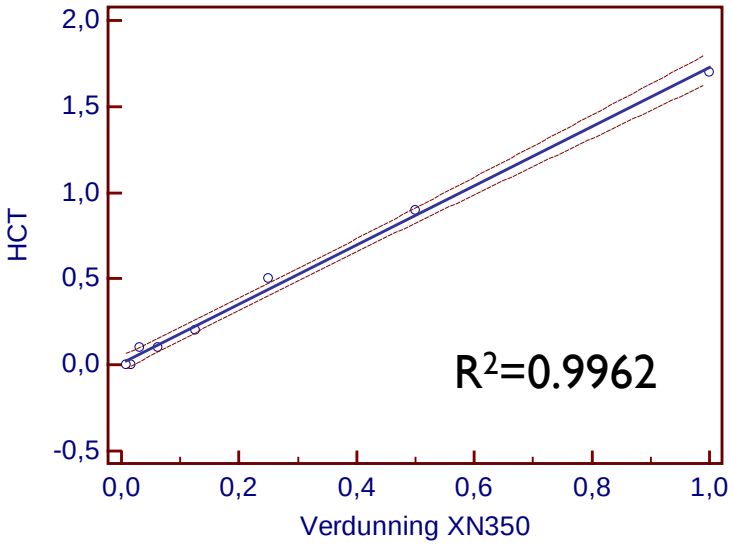
Evaluatie celtellers

Lineariteit









Evaluatie celtellers

Discussie

Correlerende hematocrieten
Verschillend MCV en RBC

	Gemeten parameters		Berekende parameters
DxH800	**(RBC*MCV)/10**	**=**	**HCT**
XN350	**(HCT*10)/RBC**	**=**	**MCV**

Evaluatie celtellers

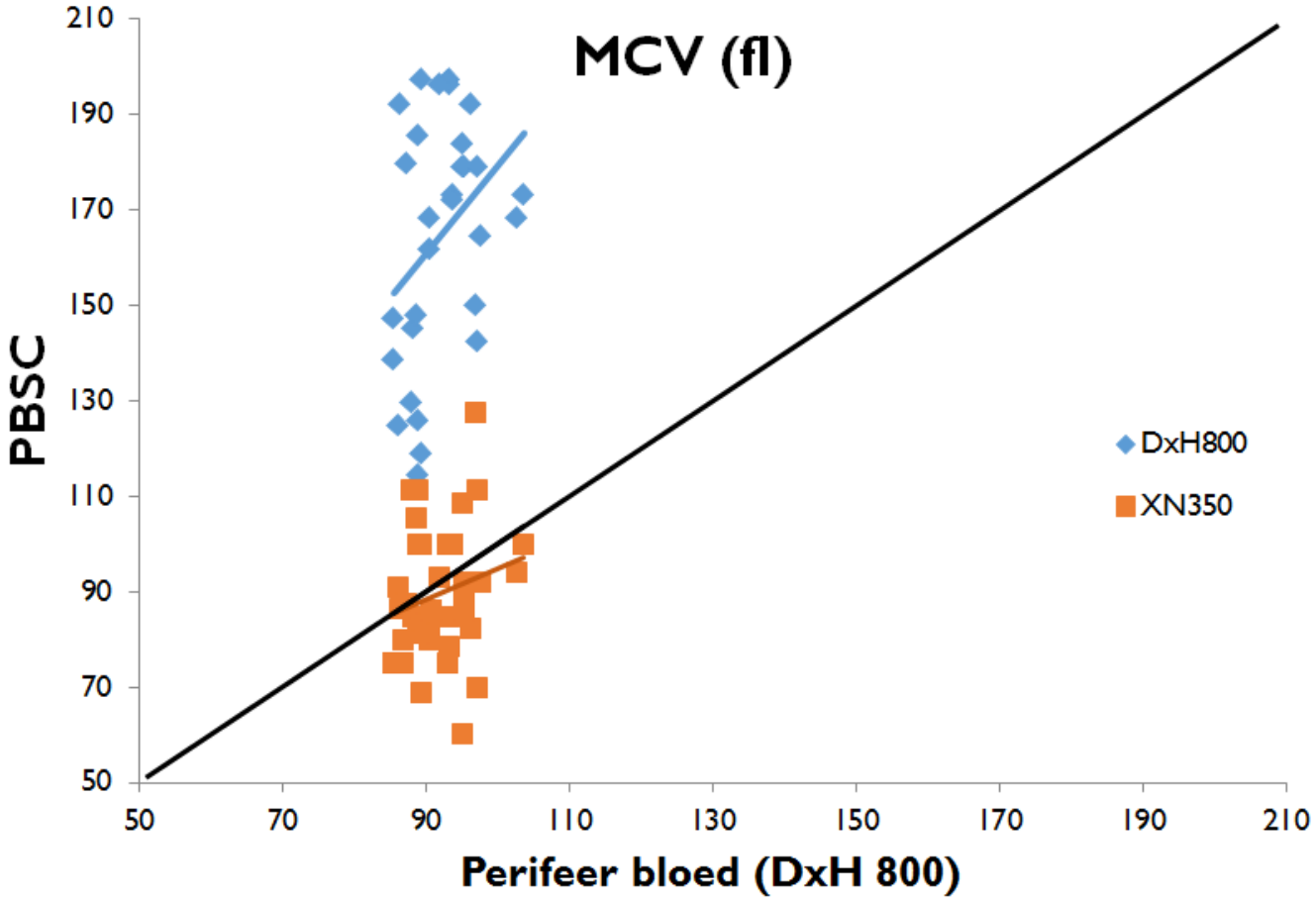
Discussie

Correlerende hematocrieten
Verschillend MCV en RBC

	Gemeten parameters		Berekende parameters
DxH 800	**(RBC*MCV)/10**	**=**	**HCT**
XN-350	**(HCT*10)/RBC**	**=**	**MCV**

Evaluatie celtellers

Discussie



Oorzaak verhoogd MCV?

Evaluatie celtellers

Oorzaken verhoogd MCV

Hypernatremie (Zandecki et al. 2007)

153,6 mmol/l (ref. 136 -145 mmol/l)

Werkelijk verhoogd MCV

Evaluatie celtellers

Oorzaken verhoogd MCV

Hypernatremie

153,6 mmol/l (ref. 136 -145 mmol/l)

Werkelijk verhoogd MCV

Leukocytose

Impedantiemeting

$RBC_{gemeten} = RBC_{werkelijk} + WBC$

Evaluatie celtellers

Oorzaken verhoogd MCV

RBC	Coulter Principle	**Red Blood Cell Count or Erythrocyte Count** • Measured directly, multiplied by the calibration factor • Corrected when WBC > 140 x 10^3 cells/µL for whole bloods. The corrected RBC = (RBC - WBC). All ranges of RBC are corrected for WBC in the body fluid cycle. • RBC = N x 10^6 cells/µL for whole blood samples; N cells/mm^3 (or cells/µL) for body fluid samples
MCV	Derived from RBC Histogram	**Mean Corpuscular Volume** • The average volume of individual erythrocytes derived from the RBC histogram, multiplied by a calibration factor • Corrected for WBC interference when WBC > 140 x 10^3 cells/µL and WBC particles were observed on the RBC histogram. • Expressed in fL.

Evaluatie celtellers
Discussie

Hematocriet

Goede correlatie tussen DxH 800 en XN-350

Overschatting t.o.v. referentiemethode

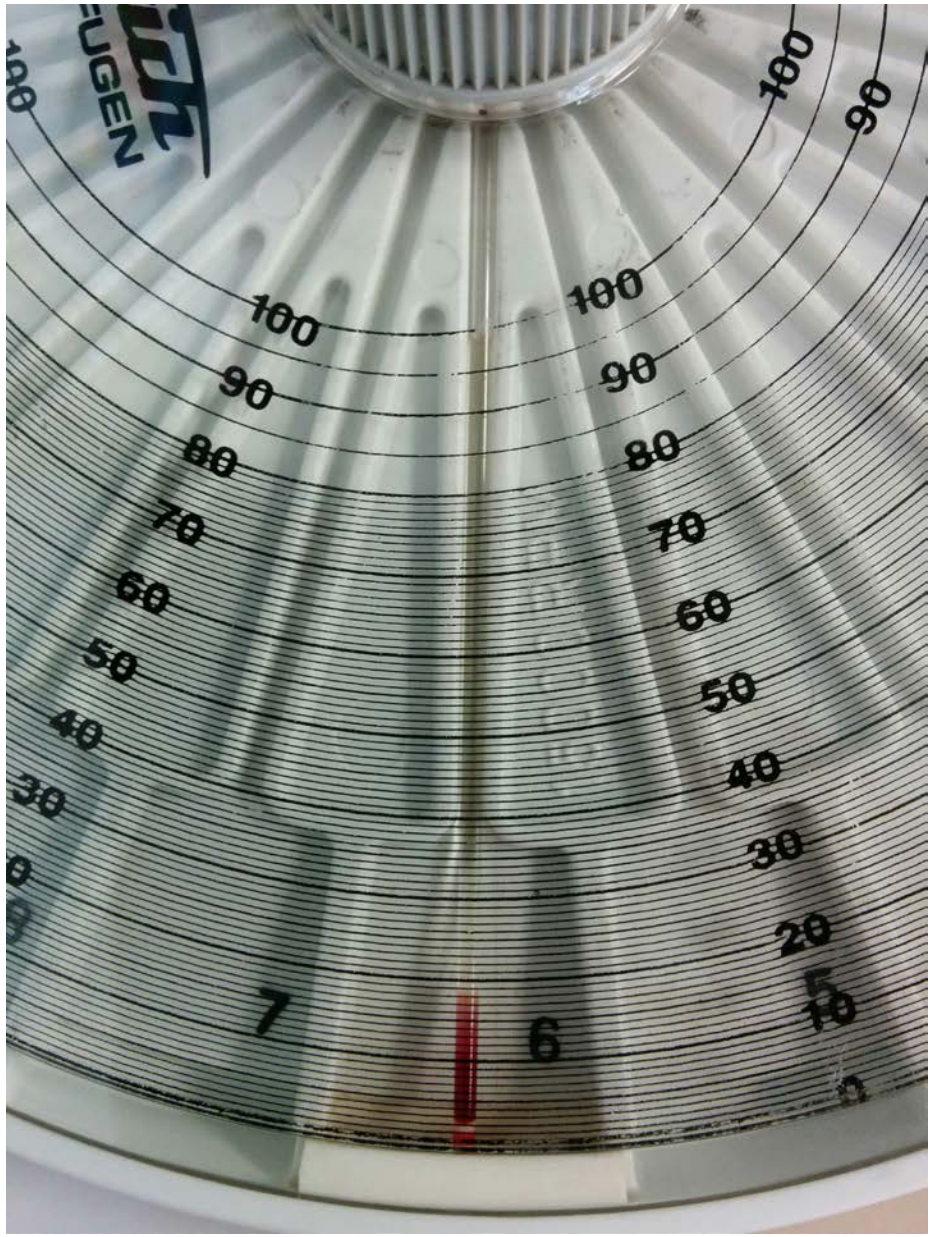
Lage resolutie

Avecilla et al.

"Comparison of manual hematocrit determinations versus automated methods for hematopoietic progenitor cell apheresis products"

TABLE 1. Descriptive statistics for various Hct determinations

				Compared to manual Hct	
Hct method	Mean	Median	SD	Mean difference	P value
Manual Hct (%)	0.99	0.50	1.55	ND	ND
Advia Hct (%)	4.14	3.80	2.35	−3.16	<0.001
Sysmex Hct (%)	3.13	2.20	2.59	−2.15	<0.001
Abbott Hct (%)	3.58	2.81	2.93	−2.59	<0.001
Coulter Hct (%)	5.44	4.70	2.67	−4.46	<0.001

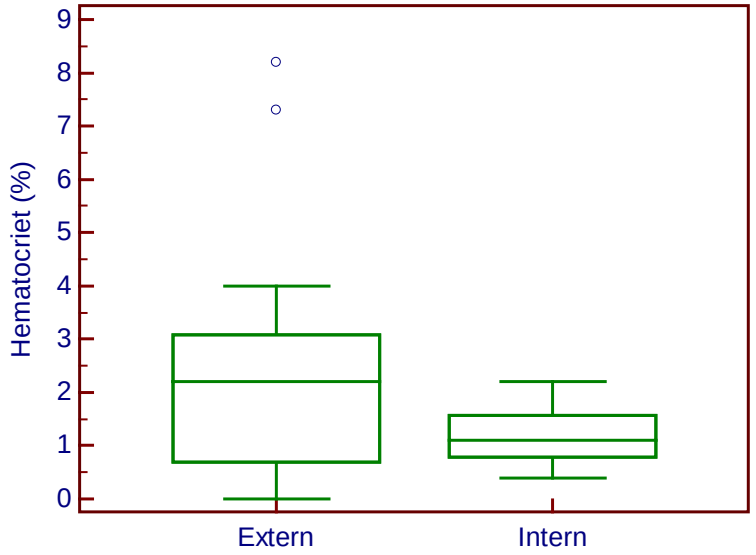


Evaluatie celtellers
Discussie

Impact licht overschatte waarde?

	Extern (n = 23)	Intern (n = 21)	Totaal (n = 44)
HCT (%)	2,4 (0,0-8,2)	1,2 (0,4-2,2)	1,9 (0,0-8,2)
RBC-volume (ml)	NB	4,5 (0,0-17,5)	NB

HCT en RBC-volume in allogene HSC-greffes verwerkt in 2015 in AZ Sint-Jan



Evaluatie celtellers

Besluit

Lage hematocrietwaarden

De hematocrieten van verschillende celtellers correleren met elkaar, ondanks sterk verschillend MCV en RBC-aantal.

Beide celtellers hebben de neiging het hematocriet te overschatten ten opzichte van de referentiemethode.

Desondanks zijn de hematocrietwaarden van de interne afereseproducten laag: lager dan de hematocrietwaarden van de extern gecollecteerde afereseproducten.