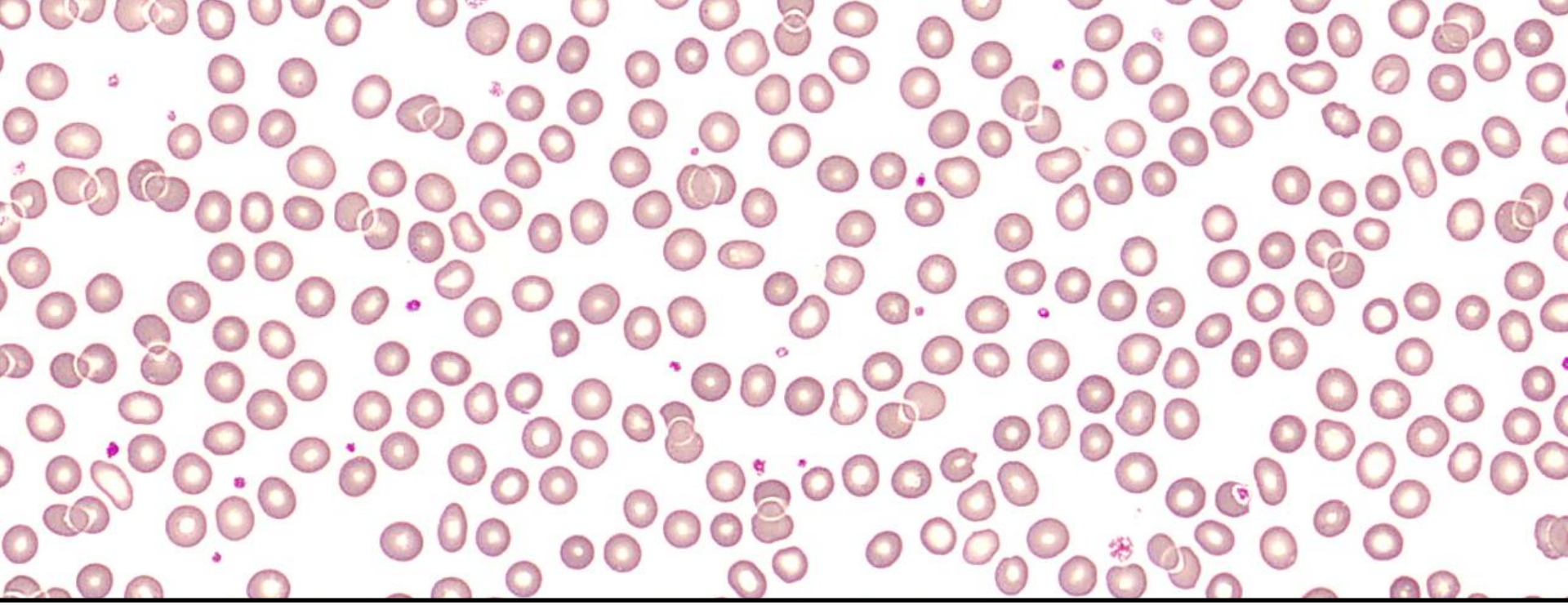


Verification and diagnostic value of the Advanced RBC Application on DI-60 (Sysmex)

26-03-2019
Lien Gruwier
Jessa hospital Hasselt

- Introduction
- Research questions
- Diagnostic value: review of literature
- Clinical utility: survey
- Verification of the Advanced RBC Application
- Conclusion and to do

Variability: grading systems and levels

RBC morphology	DI-60 default values			ICSH guidelines			UZ Leuven guidelines				Constantino 2014		
	1 (Slight)	2 (Moderate)	3 (Marked)	1 (Few)	2 (Moderate)	3 (Many)	Rare	Small excess	Medium excess	Large excess	1 (Slight/Few)	2 (Moderate)	3 (Marked)
Polychromasia	1	5	10	N/A	5	>20		0,3	>1	>2	3	6	>20
Hypochromic cells	6	25	50	N/A	11**	>20		1	>4	>12,5	5	16	>40
Microcytosis	6	25	50	N/A	11**	>20		1	>4	>12,5			
Macrocytosis	6	25	50	N/A	11**	>20		1	>4	>12,5			
Poikilocytosis	10	25	50	Report the specific cell shape									
Anisocytosis	15*	20*	25*	N/A	11**	>20							
Target cells	5	10	30	N/A	5	>20		0,6	>2	>4	5	11	>25
Schistocytes	1	3	6	<1	1	>2		0,3	>1	>2	1	6	>15
Helmet cells	1	3	6	Included in schistocyte count			Included in schistocyte count						
Sickle cells	5	10	30	N/A	1	>2		0,1	>0,3	>0,7	Report if present		
Spherocytes	1	3	6	N/A	5	>20	0,1	0,3	>1	>2	1	6	>20
Elliptocytes	6	20	50	N/A	5	>20		0,3	>1	>2	6	21	>50
Ovalocytes	6	20	50	N/A	5	>20		0,6	>2	>4			
Teardrop cells	1	3	6	N/A	5	>20		0,3	>1	>2	If > 4%: report as present		
Stomatocytes	5	10	30	N/A	5	>20		0,3	>1	>2	If > 30%: report as present		
Acanthocytes	5	10	30	N/A	5	>20	0,1	0,3	>1	>2	1	11	>30
Echinocytes	10	25	50	N/A	5	>20		0,6	>2	>4	If > 30%: report as present		
Howell-Jolly bodies	1	3	6	N/A	2	>3		0,1	>0,3	>0,7	Report if present		
Pappenheimer bodies	1	3	6	N/A	2	>3		0,1	>0,3	>0,7	Report if present		
Basophilic stippling	1	3	6	N/A	5	>20		0,1	>0,3	>0,7			
Malaria parasites	1	3	6	Report if present			Report if present				Report if present		
Anulocytes	1	1	2				0,1	0,3	>1	>2			
Pencil cells	5	5	20				0,1	0,3	>1	>2			
Rouleaux	1	1	2	Report if present			Report if present					11	>50
Agglutination	0	0	0	Report if present			Report if present				Report if present		
Dimorphism	0	0	0	Report and describe if present							Report if present		
Anisochromasia	1	3	6										
Irregularly contracted cells	0	0	0	N/A	1	>2					If > 4%: report as present		
Bite cells	0	0	0	N/A	1	>2					If > 4%: report as present		
Blister cells	0	0	0	N/A	1	>2							
Oval macrocytes				N/A	2	>5					Report if present		
Megalocytes							0,1	0,3	>1	>2			
Cabot rings								0,1	>0,3	>0,7			

N/A: not applicable

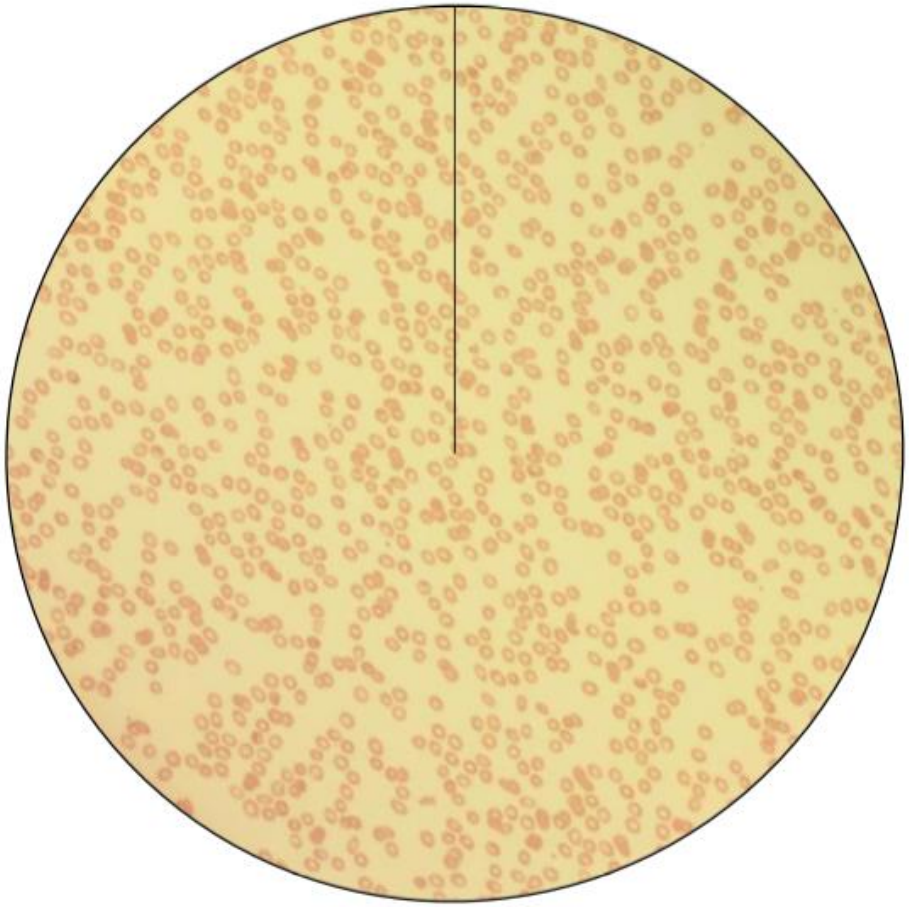
* expressed as area distribution width %

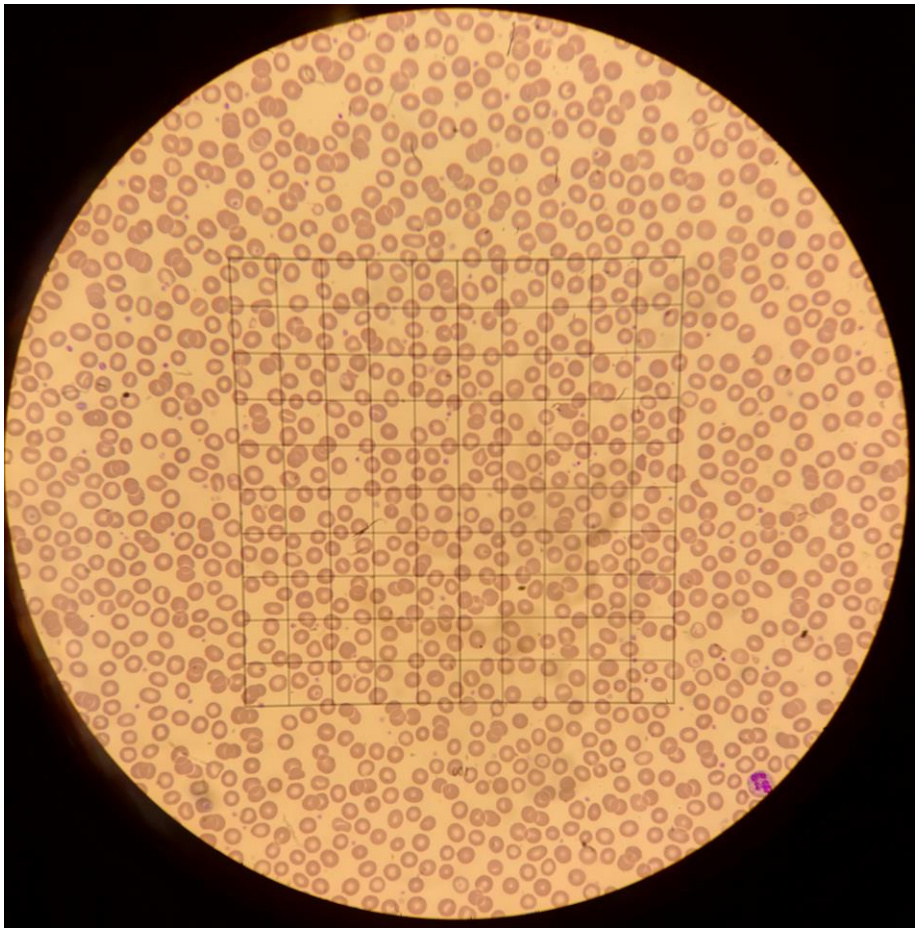
** The ICSH recommends that the analyser generated MCH, MCV and RDW be used rather than grading by visual microscopic examination, unless abnormal RDW or red cell histogram suggests the presence of macrocytes/microcytes even though the MCV is normal.

RBC morphology included in verification of Advanced RBC Application.

Not included in guideline

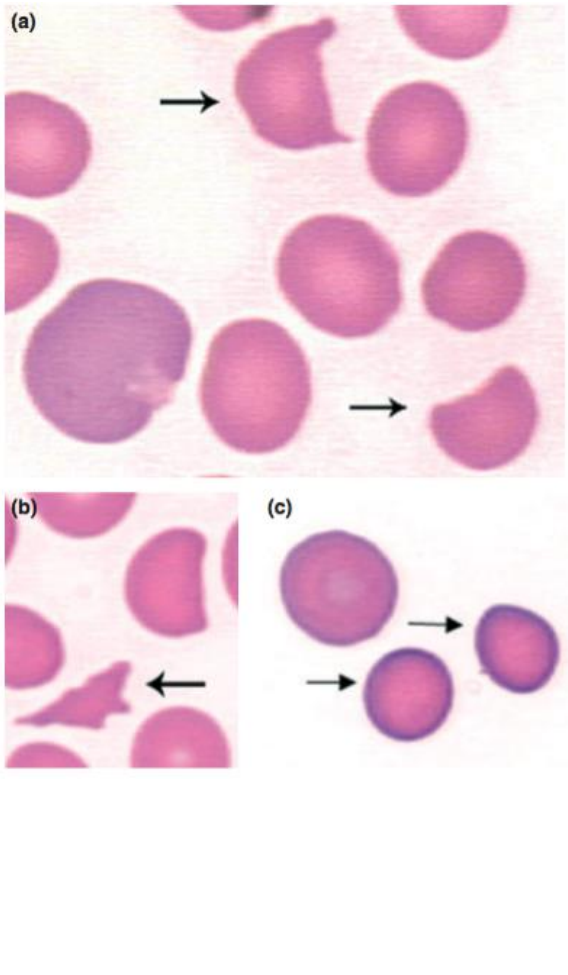
Variability: counting method

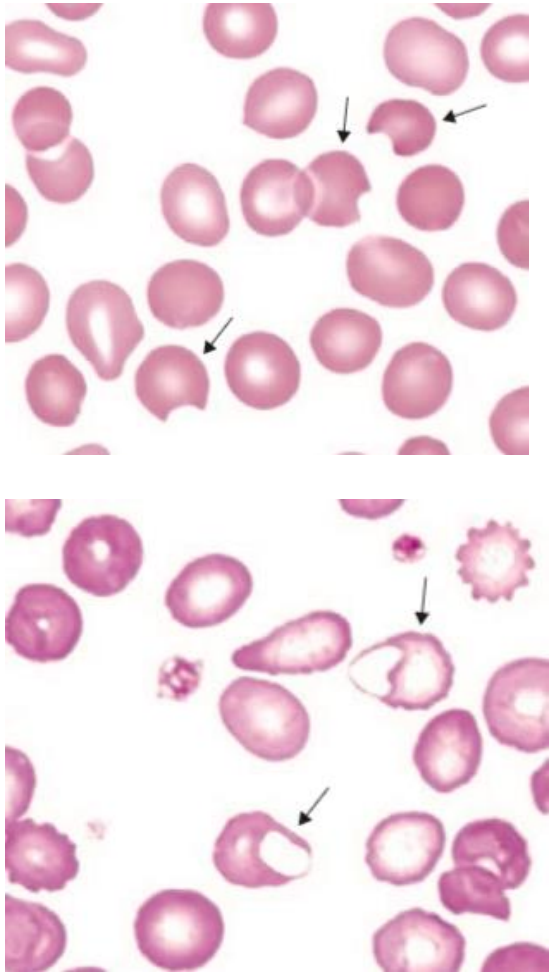


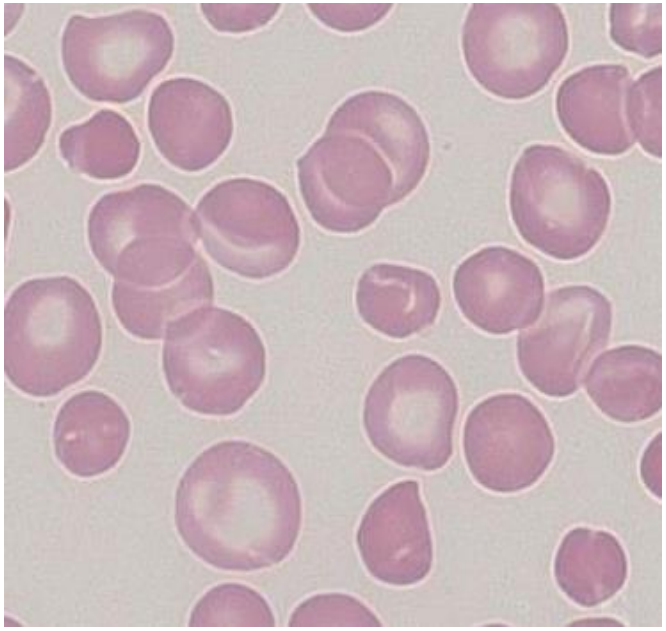


Left: Dorien Van den Bossche, Right: own collection

Variability: morphological criteria







Left: Zini et al 2011, Middle: Rodak et al 2017, Right: Palmer et al 2015

Solutions?

International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology
ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes

G. ZINI*, G. D'ONOFRIO†, C. BRIGGS‡, W. ERBER§, J. M. JOU¶, S. H. LEE**, S. MCFADDEN††, J. L. VIVES-CORRONS¶, N. YUTAKA‡‡, J. F. LESESVE§§

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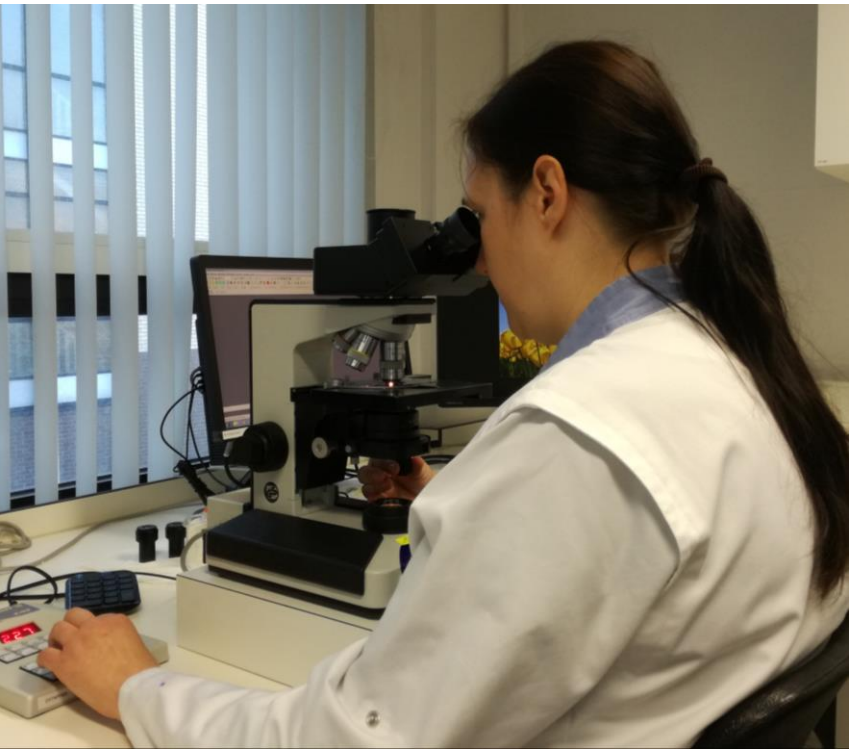
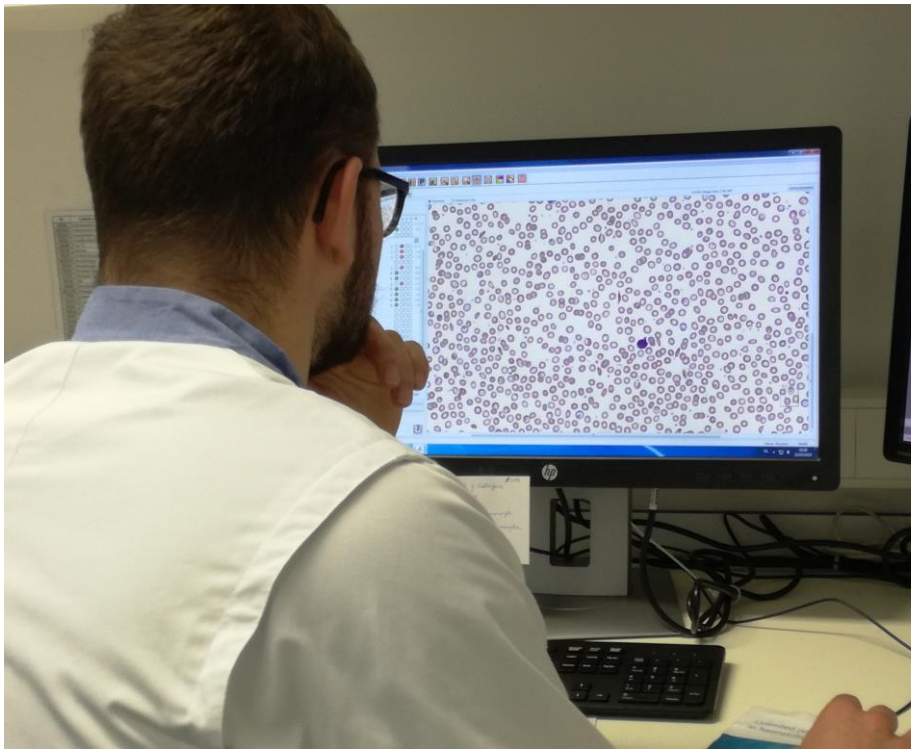
ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features

L. PALMER*, C. BRIGGS†, S. MCFADDEN‡, G. ZINI§, J. BURTHEM¶, G. ROZENBERG**, M. PROYTCHEVA††, S. J. MACHIN†

Solutions?





- Introduction
- **Research questions**
- Diagnostic value: review of literature
- Clinical utility: survey
- Verification of the Advanced RBC Application
- Conclusion and to do

1. What is the diagnostic value of red blood cell morphology abnormalities?

1. How does the clinician use and interpret RBC morphology? Do we need to change our way of reporting RBC morphology in order to provide more comprehensible and clinically relevant information?

1. How can we make use of the Advanced RBC Application on DI-60 in our laboratory?

- Introduction
- Research questions
- **Diagnostic value: review of literature**
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REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Reporting and grading of abnormal red blood cell morphology

B. T. CONSTANTINO

International Journal of Laboratory Hematology
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REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Red blood cell morphology

J. FORD

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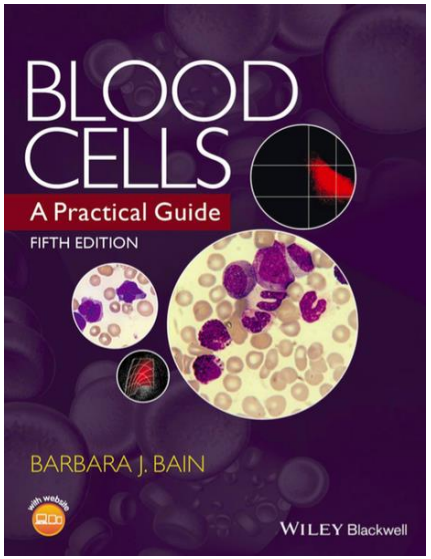
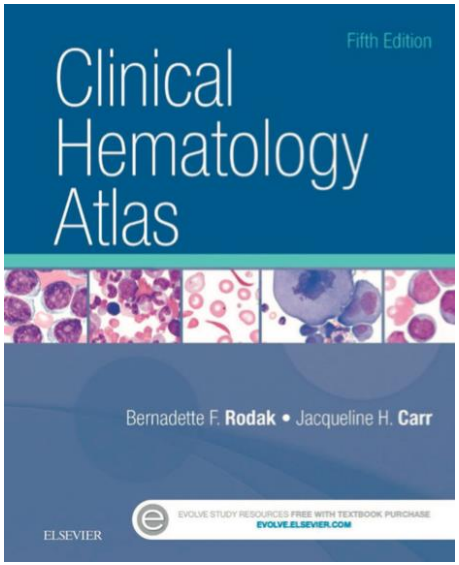
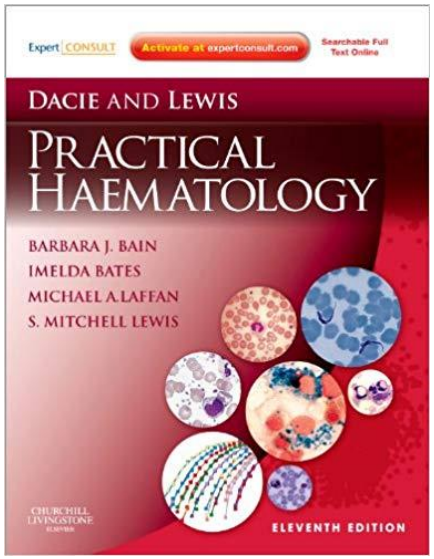
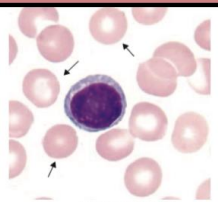
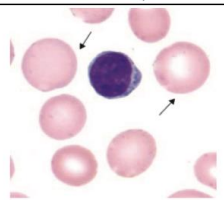
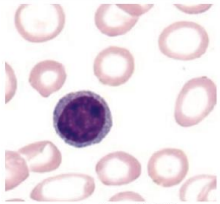
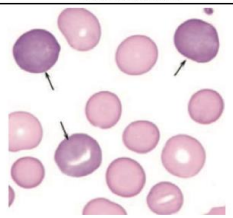


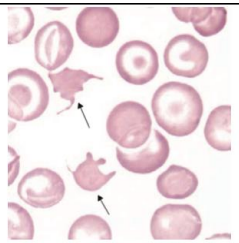
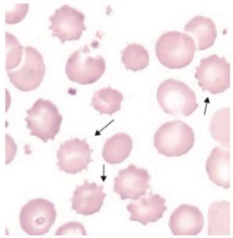
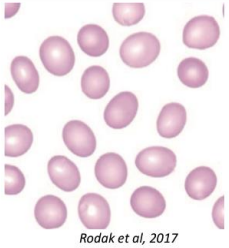
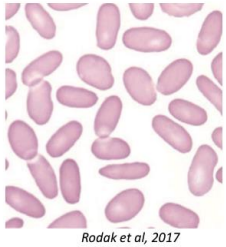




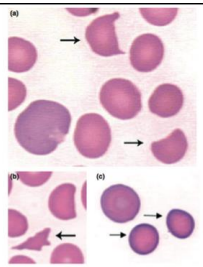
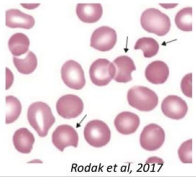
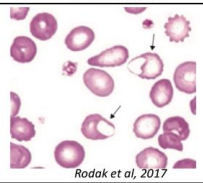
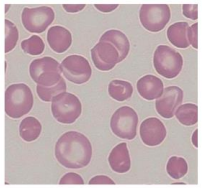
Size and color

Recommended nomenclature	Morphology	Common clinical conditions associated with
Microcytosis	 *Rodak et al, 2017*	- iron deficiency anemia - thalassemias and some other hemoglobinopathies - chronic inflammation (some cases) - lead poisoning - sideroblastic anemia - slight degree can be normal for children
Macrocytosis	 *Rodak et al, 2017*	- liver disease - hypothyroidism - vitamin B12 deficiency - folate deficiency - medication-induced: methotrexate, hydroxyurea, doxorubicine, azthioprine, mercaptopurine, fluorouracil, hydroxycarbamide, … - reticulocytosis - MDS - aplastic anemia - slight degree can be normal for neonates, pregnant women and elderly
Anisocytosis		non-specific feature of almost any blood disorder
Dimorphism		- transfusion - myelodysplastic syndromes - sideroblastic anemia - double deficiency of iron and either vitamin B12 or folic acid - early in treatment process of vitamin B12, folate, or iron deficiency
Hypochromia	 *Rodak et al, 2017*	Any of the conditions leading to microcytosis may also cause hypochromia. Slight degree can be normal for children
Polychromasia	 *Rodak et al, 2017*	- hematopoietic stress: acute and chronic hemorrhage, hemolysis, … - effective treatment of iron, vitamin B12 or folic acid deficiency - extramedullary erythropoiesis: primary myelofibrosis, metastatic carcinoma of the bone marrow, … - normal in neonates

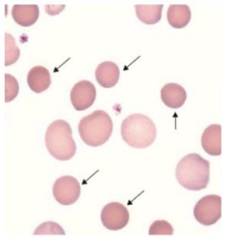
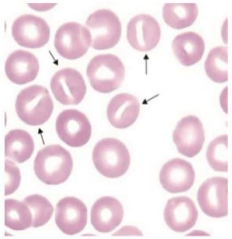
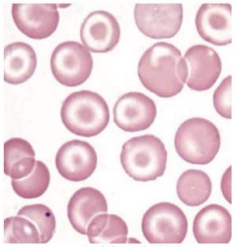
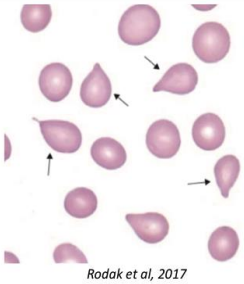
Shape

Acanthocytes	 *Rodak et al, 2017*	- advanced liver disease: alcoholic cirrhosis, severe viral hepatitis, hemochromatosis, ... - hyposplenism - anorexia nervosa, starvation and malabsorption of lipids - hypothyroidism - vit E deficiency - hereditary abetalipoproteinemia - associated with inherited degenerative neurological disease (neuroacanthocytosis): McLeod red cell phenotype, HARP syndrome, ...
Echinocytes	 *Rodak et al, 2017*	- storage artifact - liver and renal disease: described in critically ill patients with multiorgan failure, hemolytic uremic syndrome, ... - severe burn injuries - following cardiopulmonary bypass - phosphate deficiency - pyruvate kinase deficiency - premature neonates
Ovalocytes	 *Rodak et al, 2017*	- Numerous and dominant abnormality: hereditary conditions affecting the red cell cytoskeleton e.g. hereditary elliptocytosis - Small number: iron deficiency, thalassemias, primary myelofibrosis, myelodysplastic syndromes, pyruvate kinase deficiency - Macrocytic ovalocytes or oval macrocytes are characteristic of megaloblastic anemia and South-East Asian ovalocytosis and are also seen in dyserythropoiesis, e.g. in primary myelofibrosis.
Elliptocytes	 *Rodak et al, 2017*	
Poikilocytosis		non-specific feature of many blood disorders

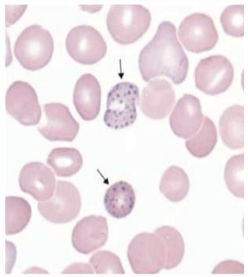
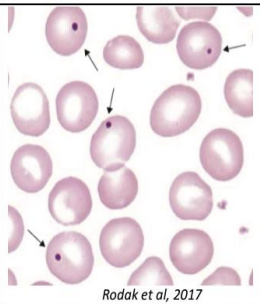
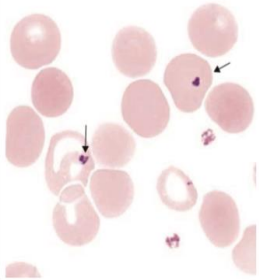
Shape

Schistocytes	 *Zini et al, 2011*	schistocytes are formed in the following situations - genetically determined disorders: thalassemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency, ... - acquired disorders of red cell formation when erythropoiesis is megaloblastic or dyserythropoietic: megaloblastic anemia, primary myelofibrosis - direct thermal injury (as in severe burns) or mechanical trauma (as in march hemoglobinuria) - as the consequence of extrinsic mechanical damage to the membrane caused by filaments of fibrin in the microvessels, increased turbulence, shear stress and RBC adhesion to abnormal endothelium (e.g. in the thrombotic microangiopathies (TMA), malfunctioning cardiac valves and cardiac assist devices, HELLP syndrome, preeclampsia, DIC, metastatic malignancy, malignant hypertension, Kasabach Merritt syndrome, …) ICSH: a schistocyte percentage > 1% in a peripheral blood smear in adults is a robust cytomorphological indication in favor of a diagnosis of TMA, when additional features suggesting an alternative diagnosis are absent.
Bite cells	 *Rodak et al, 2017*	- G6PD deficiency - hemoglobin C disease, hemoglobin C/β thalassemia, sickle cell/hemoglobin C disease, ... - unstable hemoglobins - severe oxidant stress (drugs or chemicals)
Blister cells	 *Rodak et al, 2017*	
Irregularly contracted cells	 *J. Burthem, M. Brereton (Palmer, 2015)*	
Sickle cells	 *Rodak et al, 2017*	sickle cell anemia and other forms of sickle cell disease

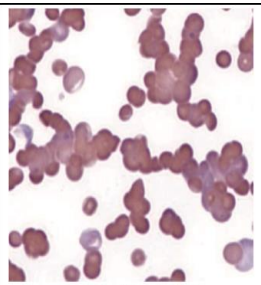
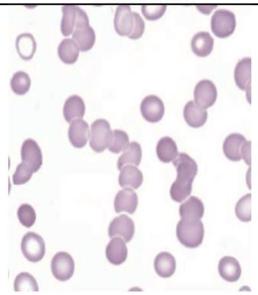
Shape

Spherocytes	 *Rodak et al, 2017*	- hereditary spherocytosis - auto-immune hemolytic anemia - ABO and Rh hemolytic disease of the fetus and newborn - drug-induced (oxidative/immune) hemolysis - delayed hemolytic transfusion reaction - severe burns
Stomatocytes	 *Rodak et al, 2017*	- alcoholism - liver disease - MDS } frequently associated macrocytosis - artifact - inherited erythrocyte membrane abnormalities: hereditary stomatocytosis, hereditary xerocytosis, South-East asian ovalocytosis (in association with ovalocytes/elliptocytes)
Target cells	 *Rodak et al, 2017*	- iron deficiency anemia - thalassemias and other hemoglobinopathies - hyposplenism - severe (obstructive) liver disease - hereditary LCAT deficiency - hereditary hypobetalipoproteinemia
Teardrop cells	 *Rodak et al, 2017*	- megaloblastic anemia - thalassemia major - myelofibrosis: primary or secondary to bone marrow infiltration

Inclusions

Basophilic stippling	 *Rodak et al, 2017*	- poisoning by lead and other heavy metals - thalassemias and other hemoglobinopathies - megaloblastic anemia - sideroblastic anemia - primary myelofibrosis - hereditary deficiency of pyrimidine 5'-nucleotidase
Howell-Jolly bodies	 *Rodak et al, 2017*	- postsplenectomy and hyposplenism - hemolytic anemia and megaloblastic anemia (especially if associated splenic atrophy) - normal in neonates
Pappenheimer bodies	 *Rodak et al, 2017*	- lead poisoning - sideroblastic anemia - postsplenectomy and hyposplenism - hemoglobinopathies
Micro-organisms		e.g. malaria parasites

Arrangement

Agglutination	 *Rodak et al, 2017*	- small agglutinates: warm auto-immune hemolytic anemia - massive agglutination: paroxysmal cold hemoglobinuria and chronic cold hemagglutinin disease
Rouleaux	 *Rodak et al, 2017*	- pregnancy - acute and chronic inflammatory disorders - plasma cell neoplasms - artifact

→ RBC morphology can be helpful in diagnostic work-up:
e.g. anemia, hemolysis, TMA, congenital diseases, …

- Introduction
- Research questions
- Diagnostic value: review of literature
- **Clinical utility: survey**
- Verification of the Advanced RBC Application
- Conclusion and to do

Enquête rode bloedcelmorfologie

Met deze enquête willen wij peilen naar de klinische relevantie en algemene kennis van rode bloedcelmorfologie bij artsen. Ons doel is om op basis van deze gegevens het rapporteren van RBC morfologische afwijkingen beter af te stemmen op de clinicus. Vul deze vragenlijst a.u.b. persoonlijk in, zonder gebruik te maken van internet of literatuur.

*Vereist

Wat is uw functie en (sub)specialisatie in dit ziekenhuis? *

Jouw antwoord

Hoe vaak bekijkt u op het rapport de MCV, MCH, MCHC of RDW?

- [ ] Altijd
- [ ] Dikwijls
- [ ] Soms
- [ ] Zelden
- [ ] Nooit

Hoe vaak bekijkt u op het rapport de rode bloedcelmorfologie (indien beschreven)?

- [ ] Altijd
- [ ] Dikwijls

Survey respondents

- 8 internal medicine residents
- 12 specialists
  - 2 hematologists
  - 4 nephrologists
  - 2 cardiologists
  - 1 pediatrician
  - 1 gynecologist
  - 1 pulmonologist
  - 1 infectious disease specialist

Hoe vaak bekijkt u op het rapport de MCV, MCH, MCHC of RDW?

- [ ] Altijd
- [ ] Dikwijls
- [ ] Soms
- [ ] Zelden
- [ ] Nooit

75% (15/20)

Hoe vaak bekijkt u op het rapport de rode bloedcelmorfologie (indien beschreven)?

- [ ] Altijd
- [ ] Dikwijls
- [ ] Soms
- [ ] Zelden
- [ ] Nooit

20% (2 nephrologists and 2 hematologists)

RBC morphology	Never significant	Only significant if many (2+ of 3+)	Significant even in small numbers (1+)	I do not know this term	Number of responses
Acanthocytes	11%	22%	22%	44%	18
Agglutination	17%	22%	28%	33%	18
Anisocytosis	12%	29%	12%	47%	17
Basophilic stippling	12%	24%	18%	47%	17
Bite cells	6%	6%	6%	83%	18
Blister cells	6%	6%	11%	78%	18
Dimorphism	6%	6%	22%	67%	18
Echinocytes	6%	11%	11%	72%	18
Elliptocyt	6%	22%	17%	56%	18
Schistocytes	17%	39%	28%	17%	18
Ghost cells	6%	11%	28%	56%	18
Howell-Jolly bodies	15%	20%	35%	30%	20
Hypochromasia	10%	45%	25%	20%	20
Irregular contracted cells	6%	6%	17%	72%	18
Macrocytosis	10%	40%	50%	0%	20
Microcytosis	10%	35%	55%	0%	20
Ovalocytes	6%	28%	17%	50%	18
Pappenheimer bodies	6%	6%	6%	83%	18
Poikilocytosis	16%	16%	37%	32%	19
Polychromasia	11%	21%	21%	47%	19
Rouleaux	11%	32%	42%	16%	19
Spherocytes	11%	42%	26%	21%	19
Stomatocytes	11%	17%	11%	61%	18
Target cells	11%	22%	33%	33%	18
Teardrop cells	22%	6%	22%	50%	18

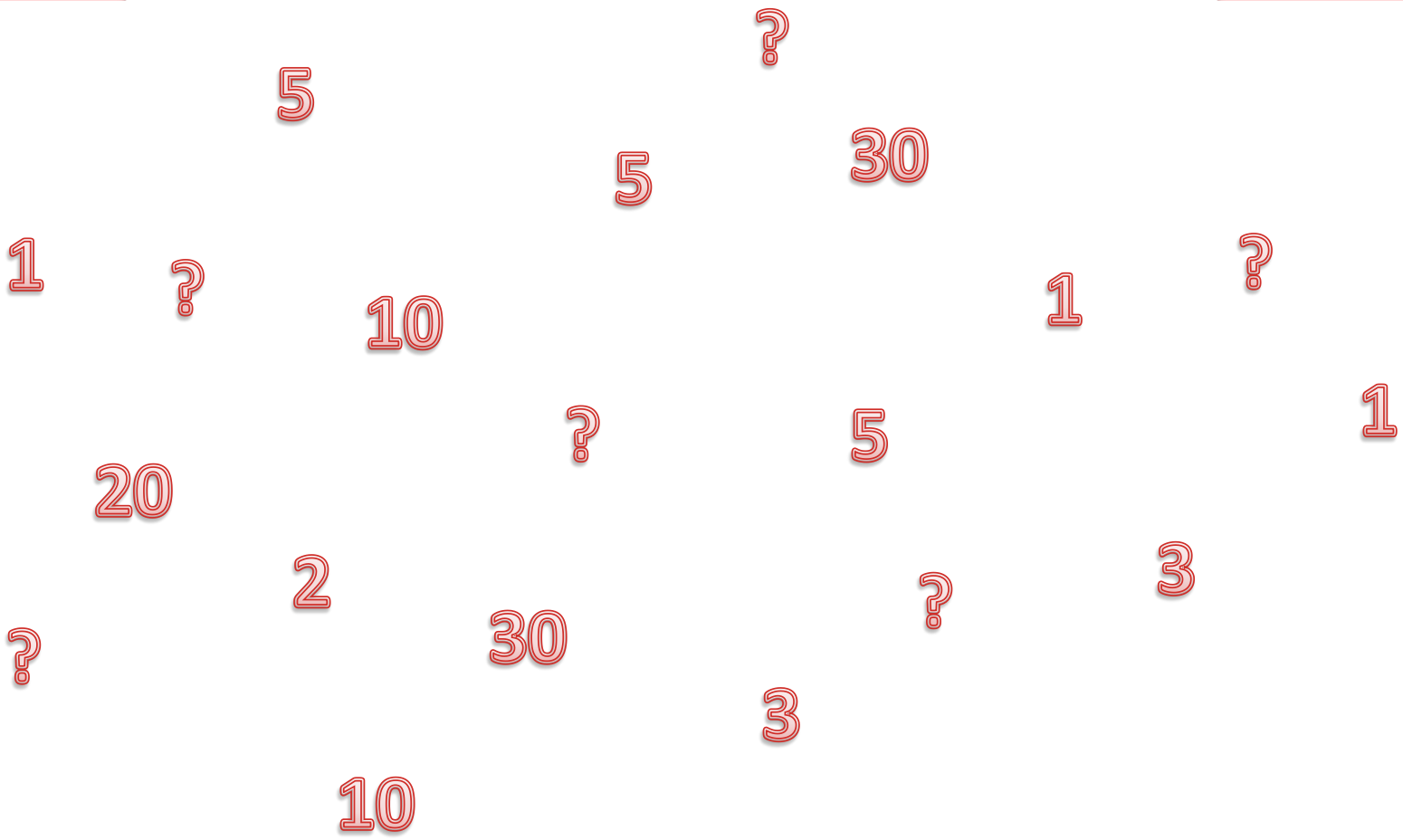
Not all responders provided answers to every question, resulting in a variable number of responses for each question.

Abnormalities of RBC morphology considered as significant (in small or large numbers) by ≥ 50%of the survey group.

RBC morphology terms unknown by ≥ 50%of the survey group.

U heeft klinisch een vermoeden van TTP (trombotische trombocytopenische purpura) en vraagt een fragmentocytentelling aan. Vanaf welk percentage fragmentocyten wordt u zekerder van uw diagnose?

Jouw antwoord



What can/should we do about this?

1. Educational initiatives
2. Modification of the laboratory report

MICROSCOPISCH ONDERZOEK

RBC morfologie	
Macrocytose	+
Anisocytose	+
Hypochromie	negatief
Polychromasie	negatief
Ovalocyten	+
Traandruppelcellen	zeldzaam
Doornappelcellen	+
Targetcellen	+++
Howell Jolly bodies	++

MICROSCOPISCH ONDERZOEK

RBC morfologie	
Targetcellen	+++
Howell Jolly bodies	++

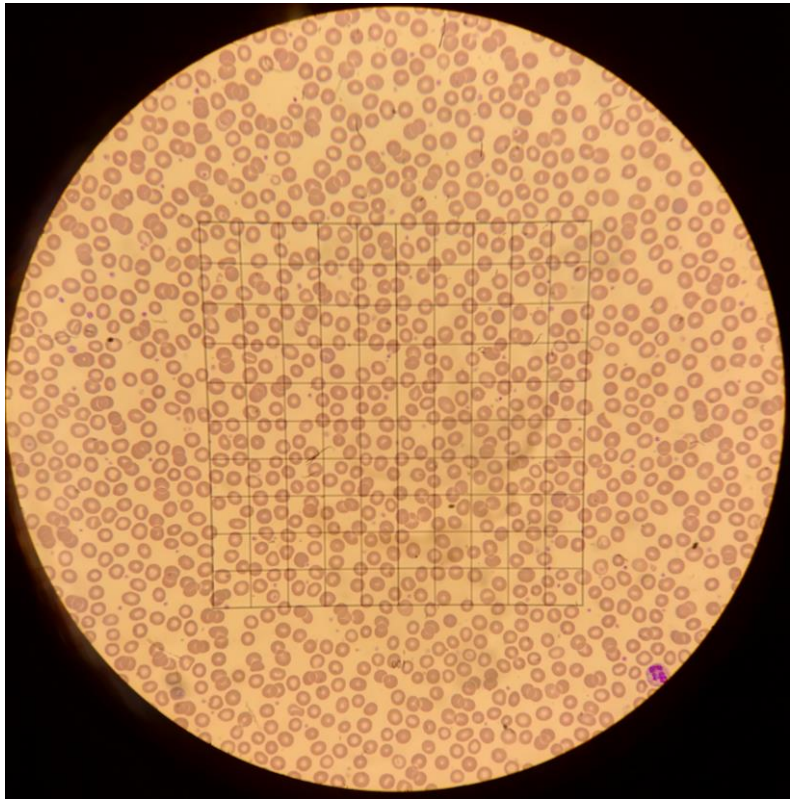
- Introduction
- Research questions
- Diagnostic value: review of literature
- Clinical utility: survey
- **Verification of the Advanced RBC Application**
- Conclusion and to do

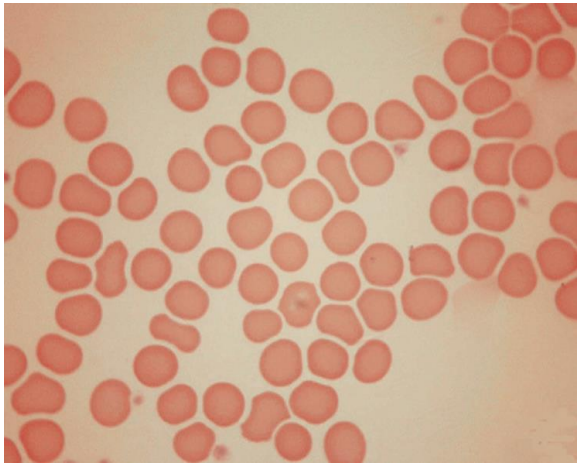
Materials and methods

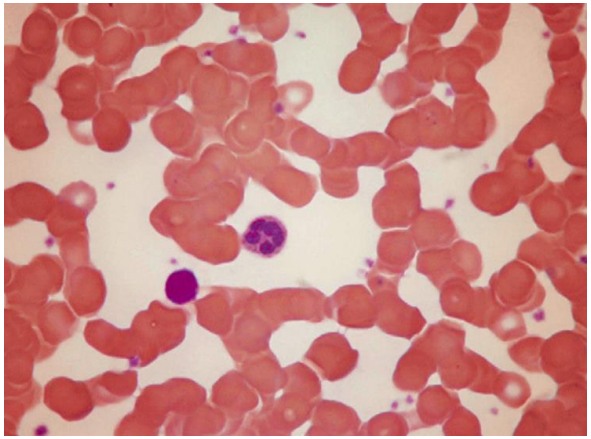
1. Samples:
   - 104 abnormal peripheral blood films
   - SP-50 May-Grünwald-Giemsa
   - All morphological categories were evaluated (negative controls)

Materials and methods

2. Manual microscopic analysis:
   = golden standard



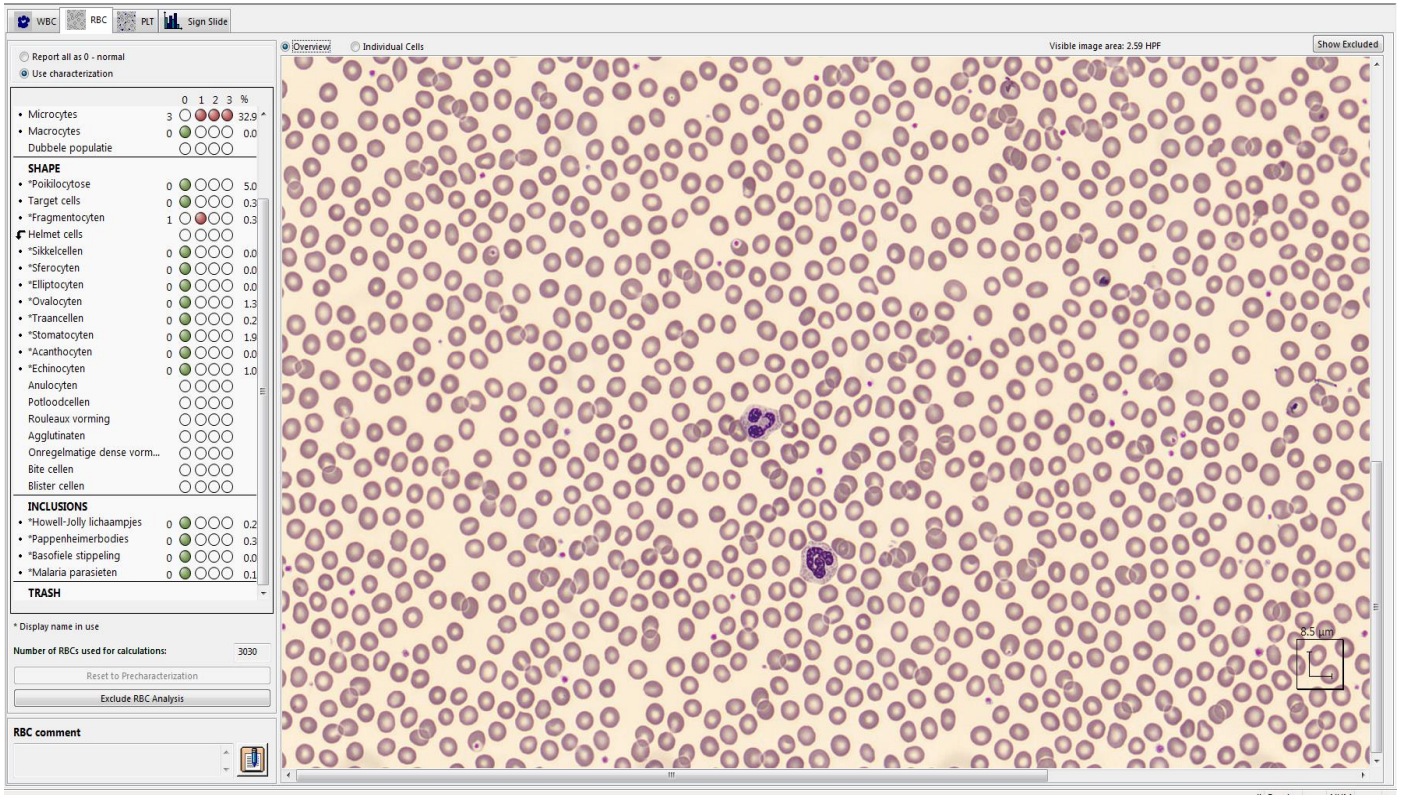




Left: own collection, Right: Bain et al 2011

Materials and methods

3. Advanced RBC Application on the DI-60 digital microscope system



Materials and methods

3. Advanced RBC Application on the DI-60 digital microscope system

Imprecision

	Within-run						Between-run					
	Few / 1+		Moderate / 2+		Many / 3+		Few / 1+		Moderate / 2+		Many / 3+	
	Mean (± SD)	CV	Mean (± SD)	CV	Mean (± SD)	CV	Mean (± SD)	CV	Mean (± SD)	CV	Mean (± SD)	CV
Polychromasia	0,32(± 0,06)	20	6,89 (± 1,20)	17	-	-	0,25(± 0,10)	39	6,51 (± 1,51)	23	-	-
Target cells	0,36 (± 0,16)	46	12,79 (± 0,81)	6	22,13 (± 2,91)	13	0,32 (± 0,09)	29	12,82 (± 0,94)	7	21,35 (± 3,09)	14
Schistocytes	0,44 (± 0,12)	27	1,72 (± 0,35)	20	7,6 (± 0,46)	6	0,96 (± 0,25)	26	1,67 (± 0,54)	33	6,91 (± 0,34)	5
Sickle cells	0,72 (± 0,17)	23	-	-	-	-	0,92 (± 0,24)	27	-	-	-	-
Spherocytes	2,97 (± 0,81)	27	-	-	-	-	2,15 (± 0,76)	36	-	-	-	-
Elliptocytes	0,11 (± 0,06)	52	6,49 (± 0,59)	9	-	-	0,05 (± 0,07)	141	6,97 (± 0,55)	8	-	-
Ovalocytes	2,16 (± 0,70)	32	10,44 (± 1,18)	11	-	-	1,5 (± 0,29)	19	8,8 (± 0,75)	9	-	-
Tear drop cells	0,35 (± 0,14)	41	10,77 (± 0,51)	5	-	-	0,35 (± 0,14)	41	10,71 (± 0,40)	4	-	-
Stomatocytes	2,18 (± 0,65)	30	9,67 (± 1,07)	11	30,57 (± 9,52)	31	1,32 (± 0,34)	26	8,45 (± 0,96)	11	32,64 (± 1,70)	5
Acanthocytes	0,07 (± 0,05)	69	9,07 (± 1,25)	14	29,53 (± 4,56)	15	0,06 (± 0,08)	141	11 (± 1,56)	14	26,02 (± 2,40)	9
Echinocytes	1,34 (± 0,48)	36	12,88 (± 2,04)	16	56,41 (± 4,71)	8	0,97 (± 0,22)	22	14,19 (± 2,81)	20	58,95 (± 6,88)	12
Howell-Jolly bodies	0,59 (± 0,12)	20	-	-	-	-	0,59 (± 0,16)	27	-	-	-	-
Pappenheimer bodies	0,30 (± 0,10)	34	2,58 (± 0,57)	22	11,78 (± 3,61)	31	0,08 (± 0,06)	79	2,25 (± 0,28)	12	11,11 (± 2,47)	22
Basophilic stippling	0,25 (± 0,24)	97	10,44 (± 1,65)	16	-	-	0,25 (± 0,07)	28	8,61 (± 1,35)	16	-	-
Malaria parasites	0 (± 0,00)	-	0,05 (± 0,05)	105	0,84 (± 0,31)	36	0 (± 0,00)	-	0,08 (± 0,04)	53	0,76 (± 0,14)	19

Method comparison

- Evaluation of degree of association:
  Pearson' correlation coefficient

- Evaluation of degree of agreement:
  Passing-Bablok regression analysis

- Detection of concentration-dependent differences:
  Bland-Altman analysis

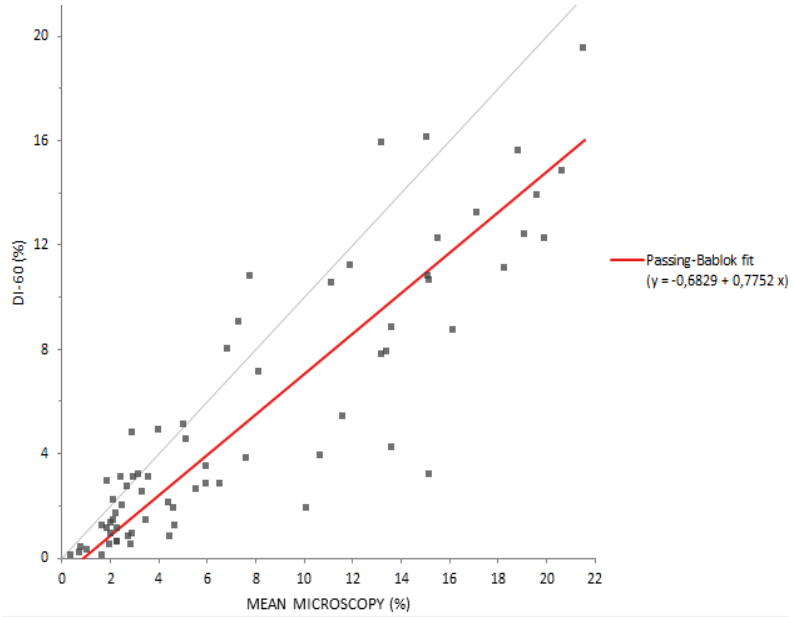
Reference values – cut-offs

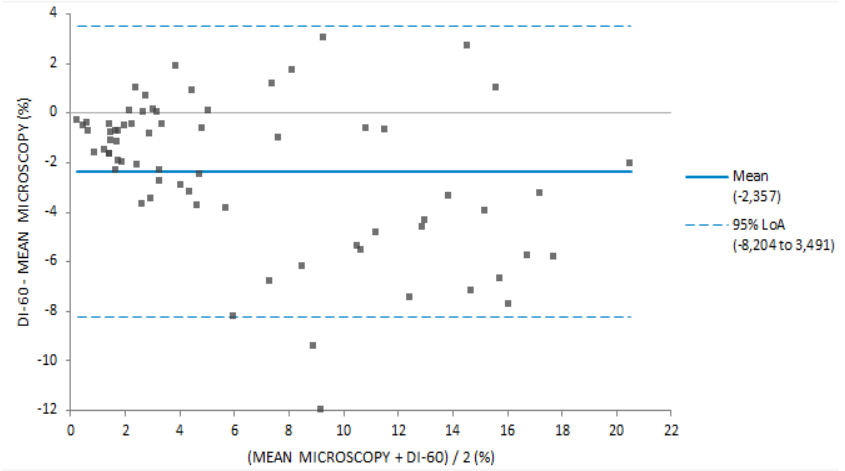
- Besides ICSH cut-offs, there are no universally accepted reference ranges

- Passing-Bablok regression analysis:
  = calculation of new cut-off values, starting from ICSH cut-offs

- If not suitable: ROC curve analysis
  = estimation of optimal cut-off for positivity

Ovalocytes





N	69

	Minimum	Maximum
MEAN MICROSCOPY	0,400	21,550
DI-60	0,100	19,500

Correlation - r	0,882

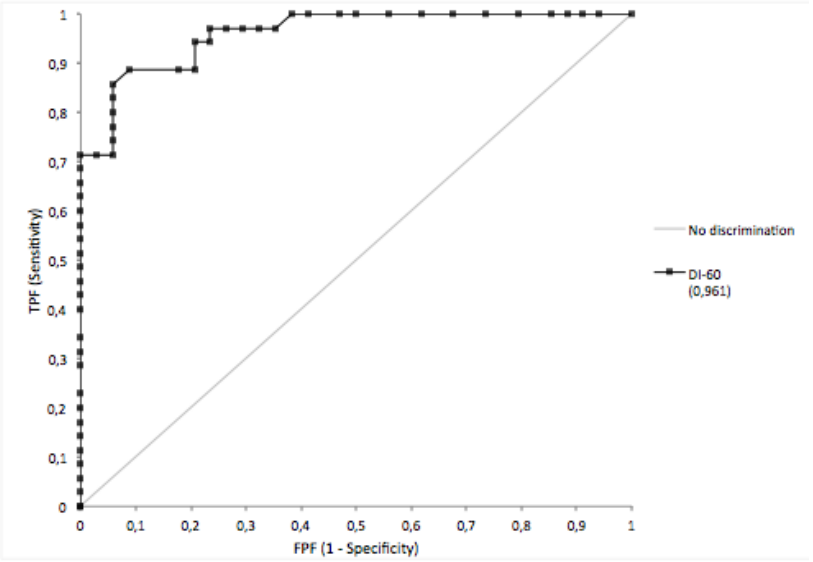
Equation	DI-60 (%) = -0,6829 + 0,7752 MEAN MICROSCOPY (%)

Parameter	Estimate	Bootstrap 95% CI
Intercept	-0,6829	-1,241 to -0,1798
Slope	0,7752	0,6740 to 0,9099

ICSH cut-off for '++' = 5% → 3,2%
ICSH cut-off for '+++' = 20% → 14,7%

Ovalocytes

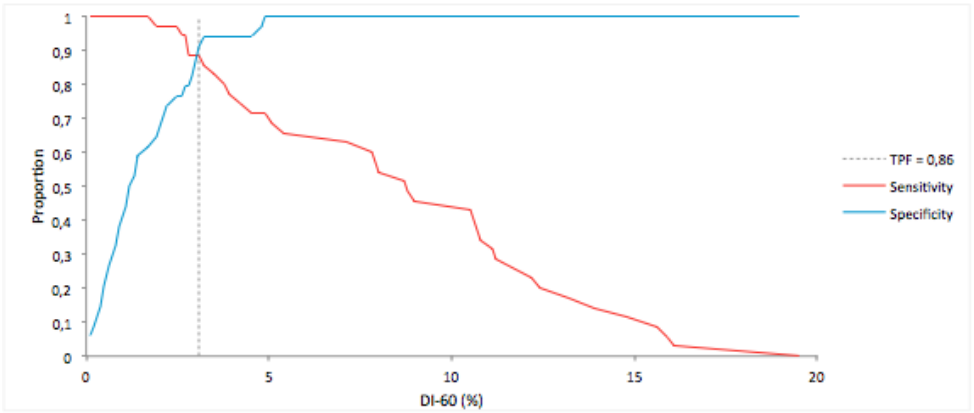
ROC Curve



	POSITIEF	NEGATIEF	Total
GEMIDDELDE	35	34	69

	AUC	95% CI	SE
DI-60	0,961	0,923 to 0,998	0,0192

Decision Threshold

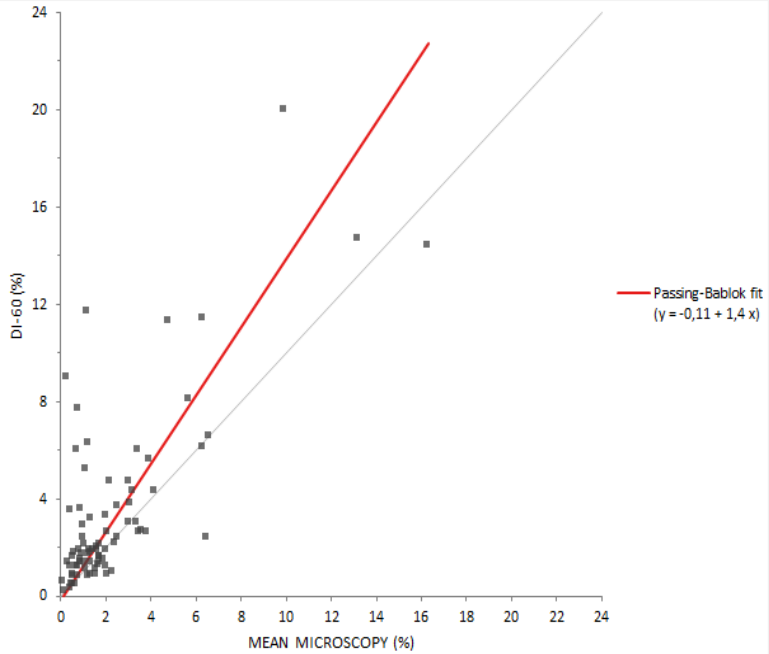


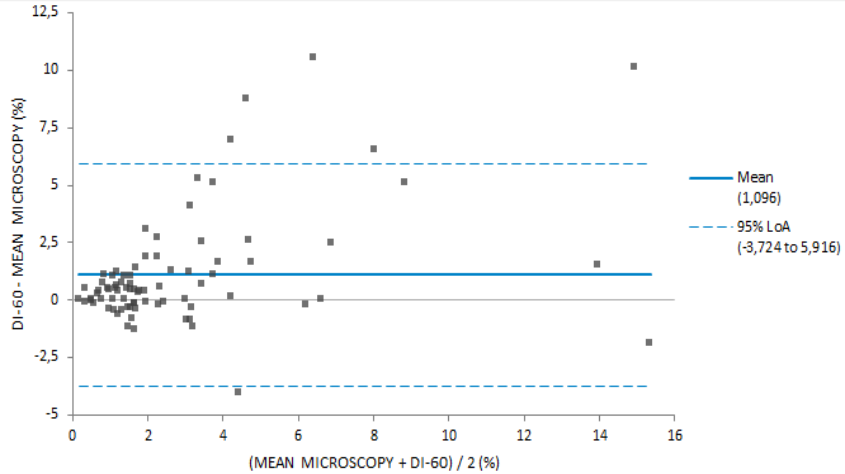
Prediction

Fixed TPF (Sensitivity)	0,86

Test	Threshold	TPF (Sensitivity)	FPF (1-Specificity)
DI-60	3,1	0,886	0,088

Schistocytes





N	80

	Minimum	Maximum
MEAN MICROSCOPY	0,100	16,300
DI-60	0,200	20,000

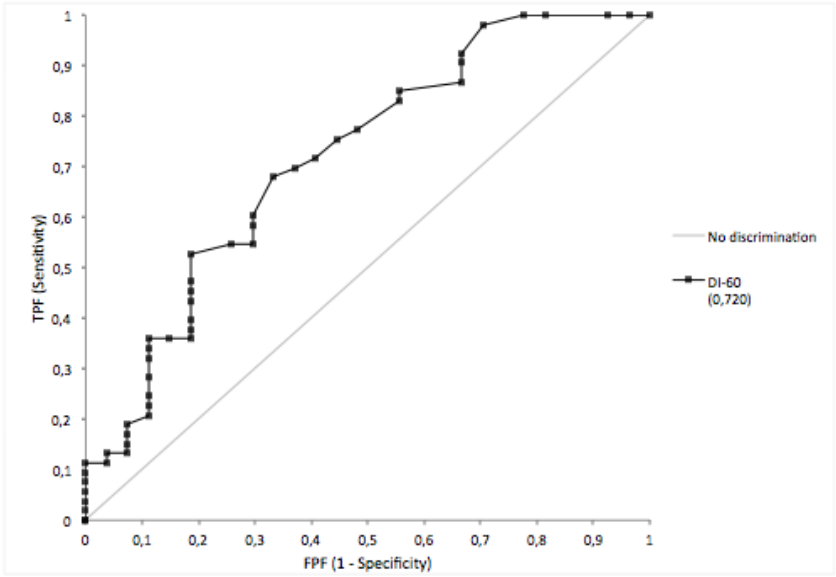
Correlation - r	0,740

Equation	DI-60 (%) = -0,11 + 1,4 MEAN MICROSCOPY (%)

Parameter	Estimate	Bootstrap 95% CI
Intercept	-0,1100	-0,6389 to 0,2374
Slope	1,400	1,083 to 1,881

Schistocytes

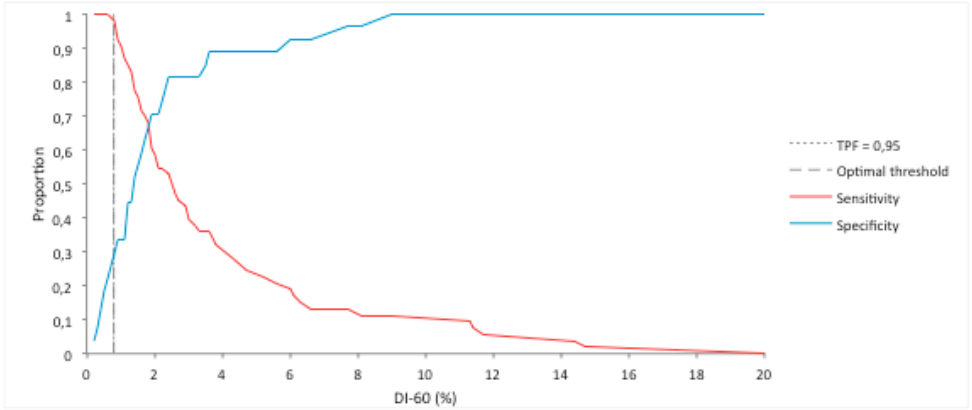
ROC Curve



	POSITIEF	NEGATIEF	Total
GEMIDDELDE	53	27	80

	AUC	95% CI	SE
DI-60	0,720	0,597 to 0,843	0,0627

Decision Threshold



Prediction

Fixed TPF (Sensitivity)	0,95

Test	Threshold	TPF	FPF
DI-60	0,8	0,981	0,704

Sensitivity / Specificity

	New settings DI-60 (%)				Sensitivity	Specificity	review by MLT?	Remarks
	negative		*positive*					
semi-quantitative	**0**	**+**	**++**	**+++**				
Acanthocytes	0	0,1	7,2	21	89%	100%	review not necessary	More high positive samples (≥ 21%) need to be included.
Basophilic stippling	0	0,1		3	-	-	review if +++ and adjust grading	No true positive samples (≥ 5%) included. Follow-up necessary.
Echinocytes	0	0,1	9,2	31	92%	98%	review not necessary	
Elliptocytes	0	0,1	3,5	15	97%	100%	review not necessary	More high positive samples (≥ 15%) need to be included.
Howell-Jolly bodies	0	0,1		0,5	100%	86%	review if +++ and adjust grading	Only 2 true positive samples (≥ 2%). Follow-up necessary. With cut-off for positivity of 0,5% there is only 44% sensitivity, but 90% specificity for splenectomy.
Ovalocytes	0	0,1	3,2	14,7	89%	91%	review not necessary	
Pappenheimer bodies	0	0,1		0,3	100%	29%	review if +++ and adjust grading	Only 3 true positive samples (≥ 2%). Follow-up necessary.
Polychromasia	0	0,1	4,1	18	100%	100%	review not necessary	More high positive samples (≥ 18%) need to be included.
Spherocytes	0	0,1		0,3	100%	92%	review if +++ and adjust grading	
Sickle cells	0			0,1	100%	52%	review if +++ and adjust grading	Only 3 true positive sample (diagnosis of sickle cell anemia). Follow-up necessary.
Stomatocytes	0	0,1		12,7	100%	74%	review if +++ and adjust grading	
Target cells	0	0,1	3,3	19,8	100%	89%	review not necessary	
Teardrop cells	0	0,1		3,8	100%	79%	review if +++ and adjust grading	Only 3 true positive samples (≥ 5%). Follow-up necessary.
	negative	*positive*						
semi-quantitative	**0**	**+**	**++**	**+++**				
Schistocytes	0	0,5		0,9	98%	30%	review if +++ and adjust grading	
Malaria parasites	0			0,1	100%	80%	review if +++ and adjust grading	Only 4 true positive samples (diagnosis of malaria infection). Low positive samples have between-run variability of 53%, therefore sensitivity is probably lower than reported. Follow-up necessary.

Other categories?

- Hypochromia, microcytosis, macrocytosis, anisochromasia and anisocytosis:
  preferable use of analyzer parameters

- Poikilocytosis: not recommended by ICSH
  → report specific cell shape

- Rouleaux, agglutination, irregular contracted cells, bite cells, blister cells and intracellular hemoglobin crystals:
  not included in application software
  → manual screening remains necessary

- Introduction
- Research questions
- Diagnostic value: review of literature
- Clinical utility: survey
- Verification of the Advanced RBC Application
- **Conclusion and to do**

Conclusion

1. RBC morphology can be helpful in diagnostic work-up: e.g. anemia, hemolysis, TMA, congenital diseases, …

2. Only a minority of hospital physicians is aware of this clinical utility.
   → educational initiatives
   → modification of the laboratory report

3. Advanced RBC Application: good sensitivity and reproducibility is possible (with adjusted cut-offs), but verification remains necessary for most categories

To do

1. More (high) positive samples need to be analyzed to further determine the utility of the Advanced RBC Application.

2. We need large multi-center studies to define grading levels for RBC morphology abnormalities that are not based on consensus (like those provided by the ICSH), but on clinical relevance (the condition of the patient: e.g. AIHA, TTP, thalassemia, ...).

3. For schistocytes, it could be useful to combine the automated FRC count with the count of the Advanced RBC Application as a screening method, but this needs to be further evaluated.

Questions?

Special thanks to our MLTs:
Kristel, Ingrid, Roel, Sigrid,
Marleen and Matthijs

1) *Guidelines and Recommendations (most recent topics on top)*

1. Palmer, L., Briggs, C., McFadden, S., Zini, G., Burthem, J., & Rozenberg, G. et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. International Journal Of Laboratory Hematology. 2015; 37(3): 287-303.
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2) *Reviews*

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4. Ford, J. Red blood cell morphology. International Journal Of Laboratory Hematology. 2013; 35(3), 351-357.

3) *Original Articles*

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5) Posters, "grey literature", presentations
17. Brusselmans C. Cytologie perifeer bloed: richtlijnen analyse cytologisch onderzoek. Retrieved from: https://w1.uzleuven.be/labo/Leermodule/HEMATO_ATLAS/pagina_extra/perifeer_bloed_algemeen_overzicht.htm