Anémies hémolytiques héréditaires

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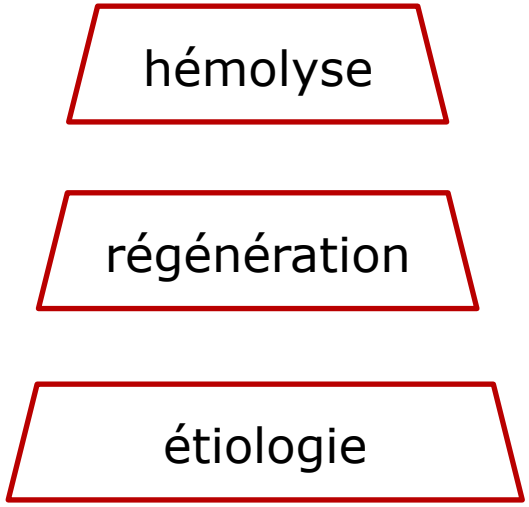
Plan

LHUB-ULB

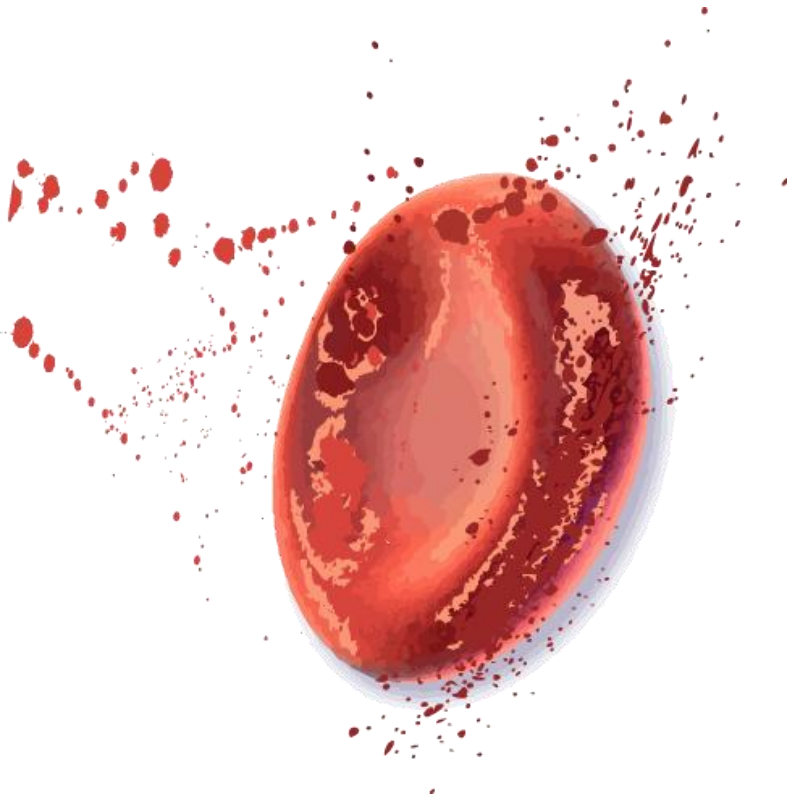
1. **Hémolyse**
2. Hémoglobinopathies
3. Enzymopathies érythrocytaires
4. Membranopathies

Démarche diagnostique

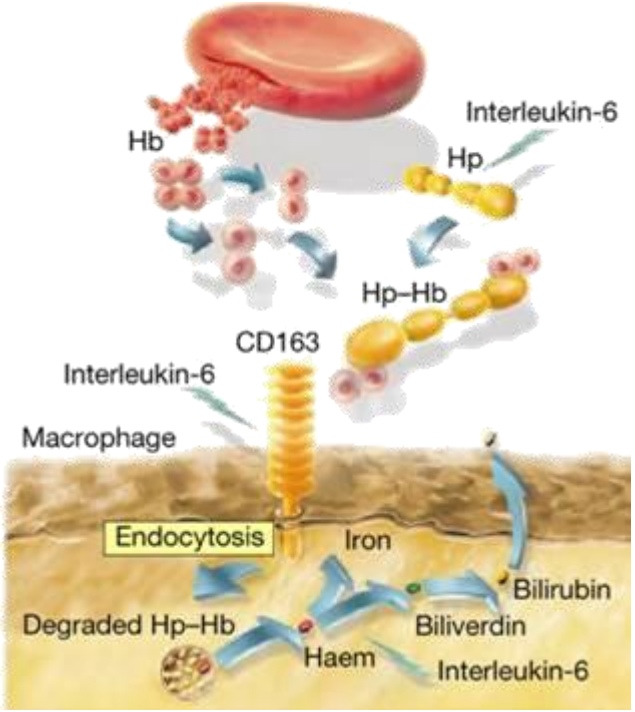


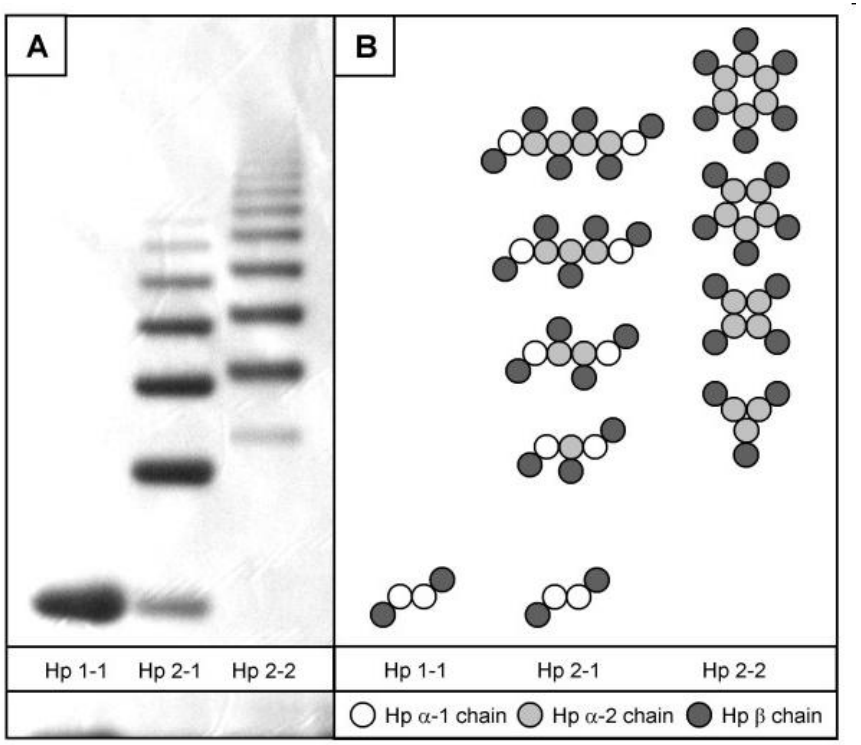
Hémolyse

1. marqueurs directs
   - LDH
   - K^+
   - hémoglobine libre
   - hémoglobine urinaire
2. marqueurs indirects
   - bilirubine non conjuguée
   - haptoglobine
   - hémopexine
3. marqueurs cellulaires
   - érythrocytaires
   - réticulocytaires



Haptoglobine





- capacité: 500-1500 mg/L Hb
- hétérogénéité phénotypique et fonctionnelle: $(\alpha\beta)_2$: α^1 - α^2

Haptoglobine

Clinical Chemistry 42:10
1589-1600 (1996)

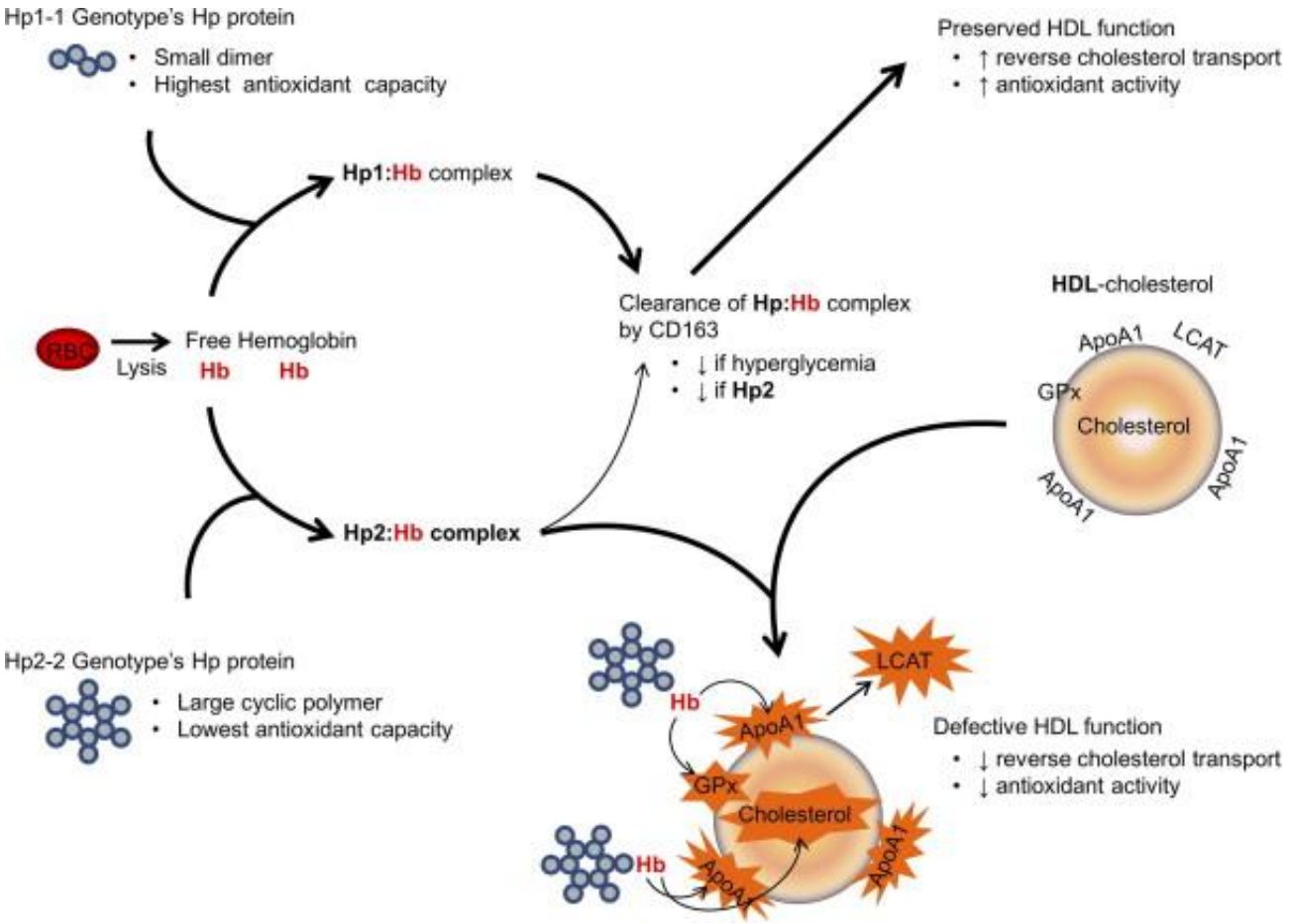
Biological and clinical significance of haptoglobin polymorphism in humans

MICHEL R. LANGLOIS and JORIS R. DELANGHE*

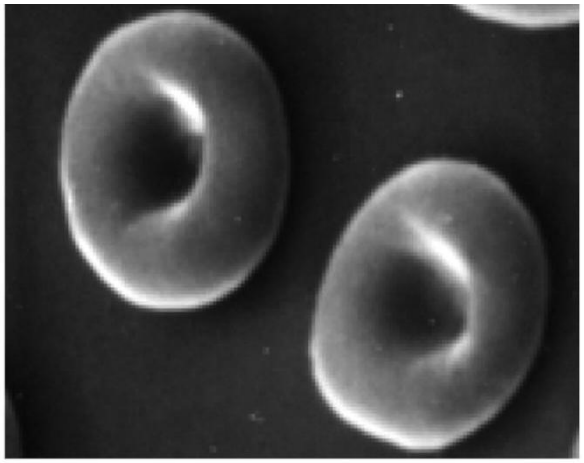
Table 1. Physical properties and reference values of Hp phenotypes.

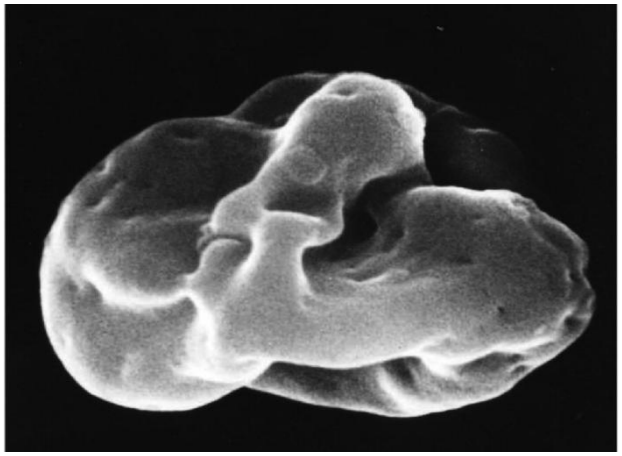
	Hp 1-1	Hp 2-1	Hp 2-2
Structural formula	$(\alpha^1\beta)_2$	$[(\alpha^1\beta)_2 + (\alpha^2\beta)_n]$ (n = 0, 1, 2, . . .)	$(\alpha^2\beta)_n$ (n = 3, 4, 5, . . .)
Apparent molecular mass, kDa	86	86–300	170–900
Reference range in serum, g/L	0.57–2.27	0.44–1.83	0.38–1.50

Haptoglobine

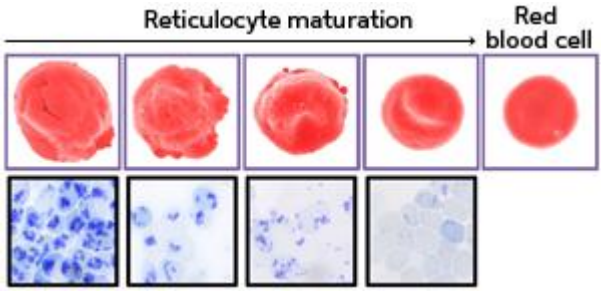


Réticulocytes



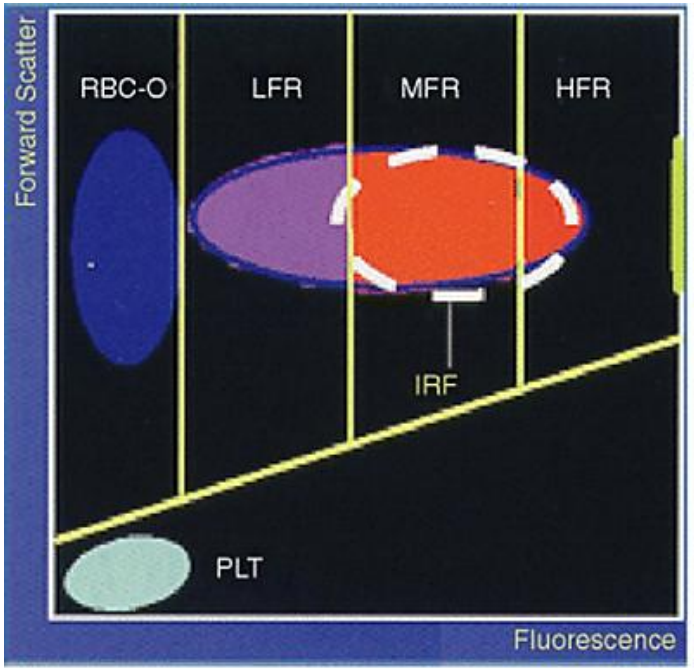


Réticulocytes vs érythrocytes	
MCV	120%
MCH	105%
MCHC	83%
Enzymes	100-200%



Réticulocytes

- nombre absolu
- adéquation avec la sévérité de l'anémie
- indices réticulocytaires
  - contenu en Hb
    - CHr
    - Ret-He
    - MCHr
    - RSf
  - volume
    - MRV
    - MCVr
  - âge: IRF



Réticulocytes

Authors,[Ref.] Year	Reticulocytes and Parameters	Company/Instrument				
		CELL-DYN 4000	GEN*S	PENTRA 120	H*3	SE 9500
d'Onofrio et al,[17] 1995 (N = 64)	MCVr (fL) CHr (pg)				103.2–126.3 25.9–30.6	
Van den Bossche et al,[73] 2002 (F: N = 175; M: N = 142)	Reticulocytes (10^9/L) IRF	F 21–98 M 30–110 0.14–0.35	F 24–73 M 30–90	F 22–95 M 31–130	F 19–64 M 2–69	F 16–66 M 16–70
			LH 750		**ADVIA 120**	**XE 2100**
GdS-Simel-Emat, 2003 (N = 127)[a]	Reticulocytes (10^9/L) IRF MCVr (fL) CHr (pg)	29–146 0.16–0.42	18–117 0.19–0.43 91–111	30–148 0.07–0.19 98–120	30–111 0.04–0.23 100–114 28.0–35.1	18–104 0.01–0.13

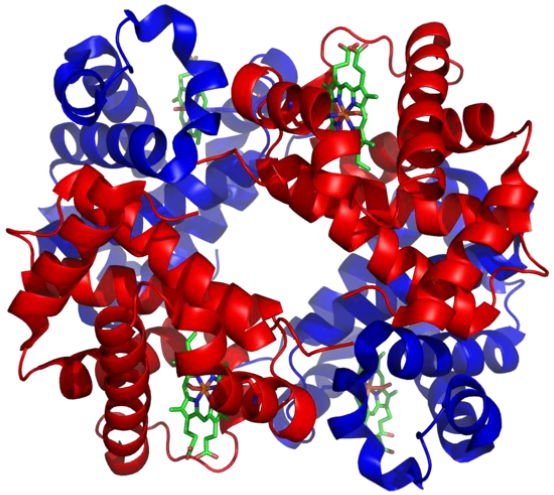
Clin Lab Med 2015;35:133

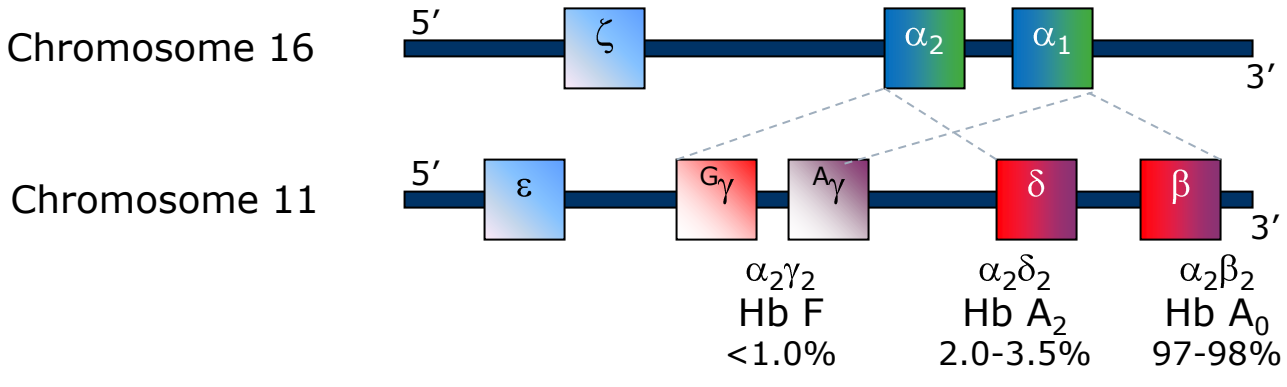
Plan

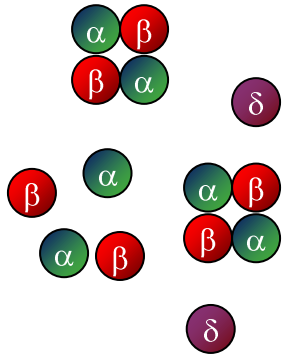
1. Hémolyse
2. **Hémoglobinopathies**
3. Enzymopathies érythrocytaires
4. Membranopathies

Hémoglobinopathies

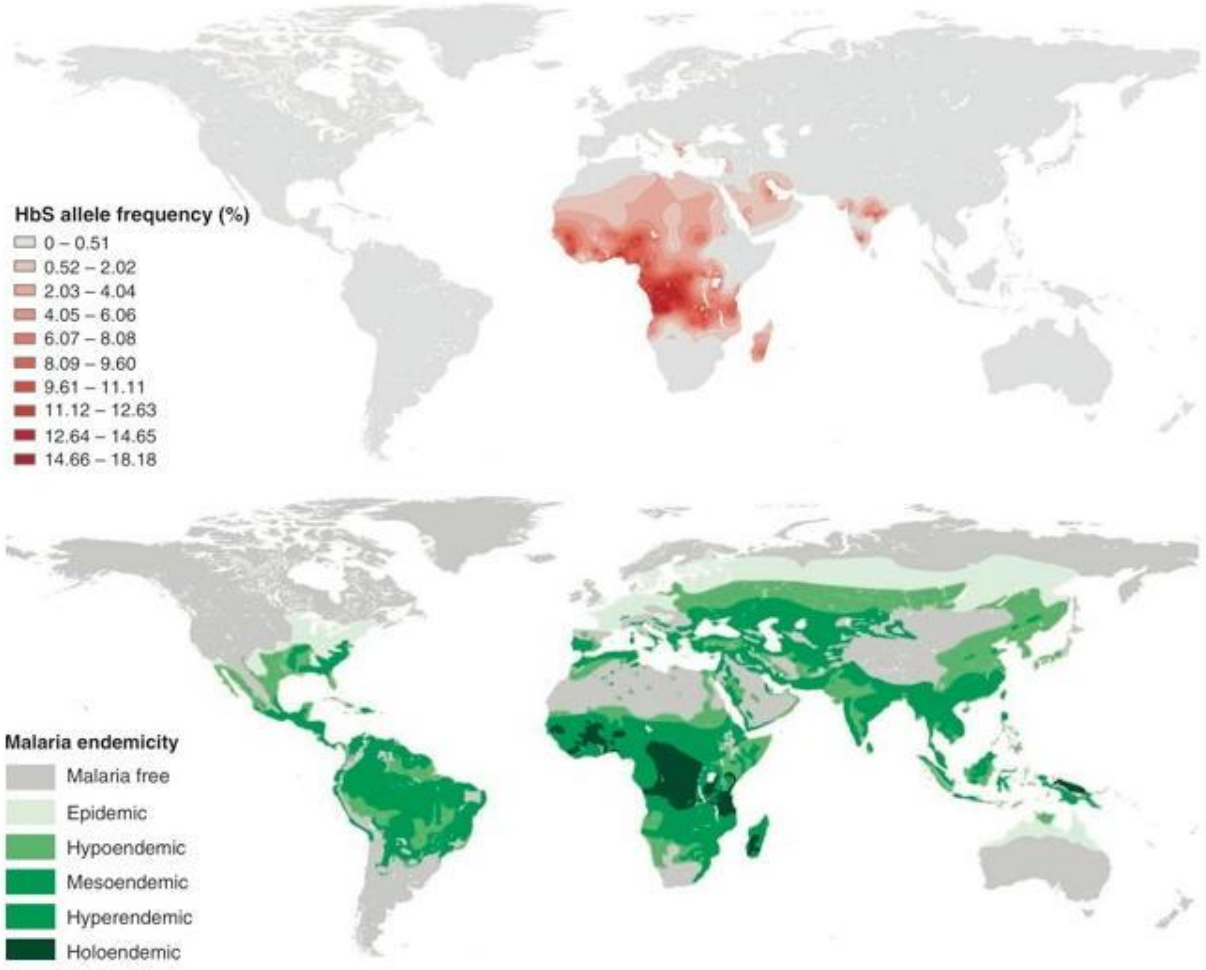
- anomalies quantitatives
  - β-thalassémies
  - α-thalassémies
- anomalies qualitatives
  - syndromes drépanocytaires
  - autres







Hémoglobinopathies



Hémoglobinopathies

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CARTE DES TÉLÉSPECTATEURS DE SECRET STORY

CARTE DU VOTE FN

Thalassémies

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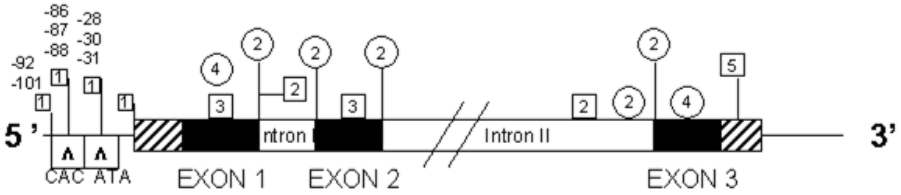
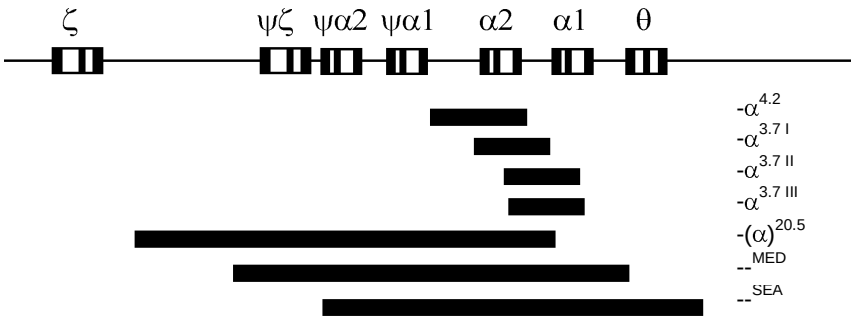
β - mutations ponctuelles: β^0, β^+

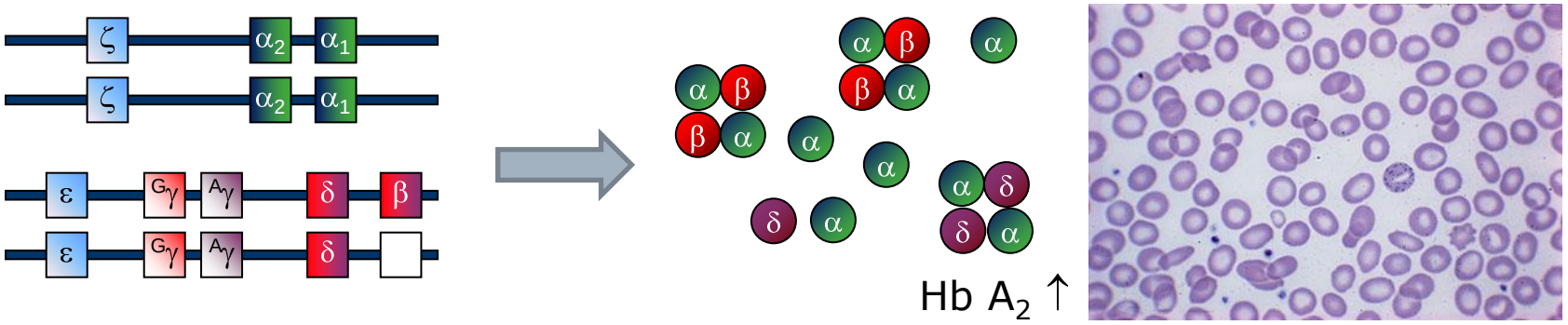


α - délétions: simples, doubles

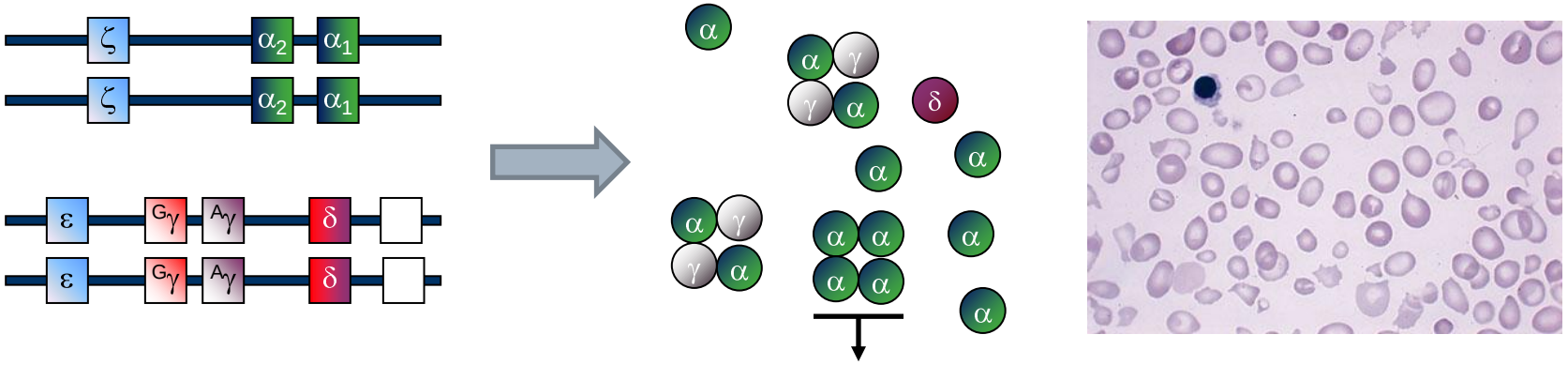


β-Thalassémie

β-thalassémie mineure

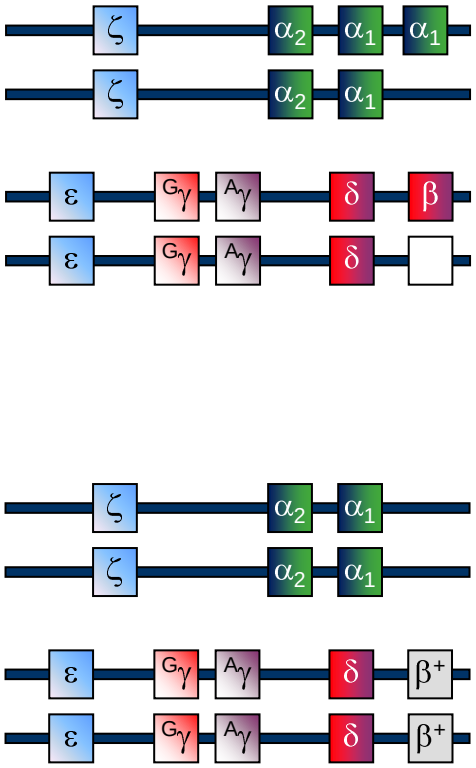


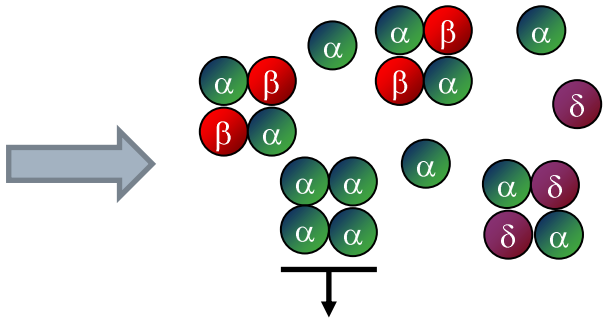
β-thalassémie majeure

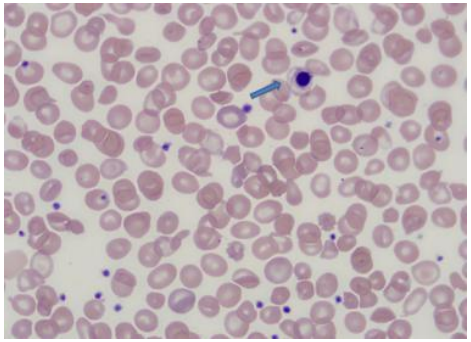


β-Thalassémie

β-thalassémie intermédiaire







α-Thalassémie

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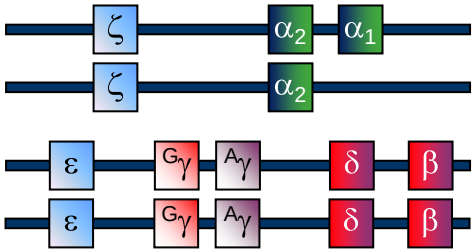
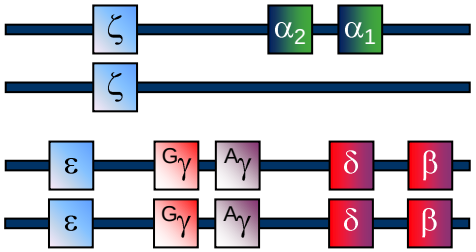
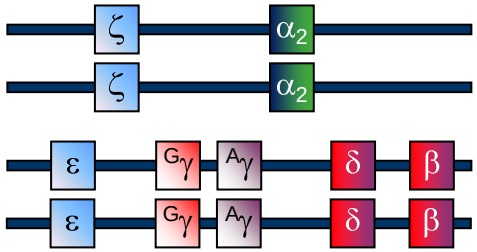
α-thalassémies "mineures"

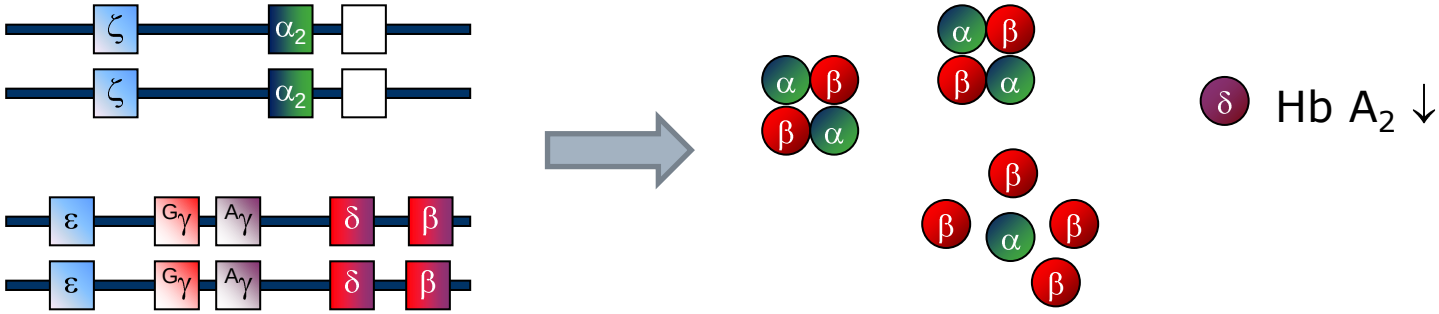


α-thalassémie 2





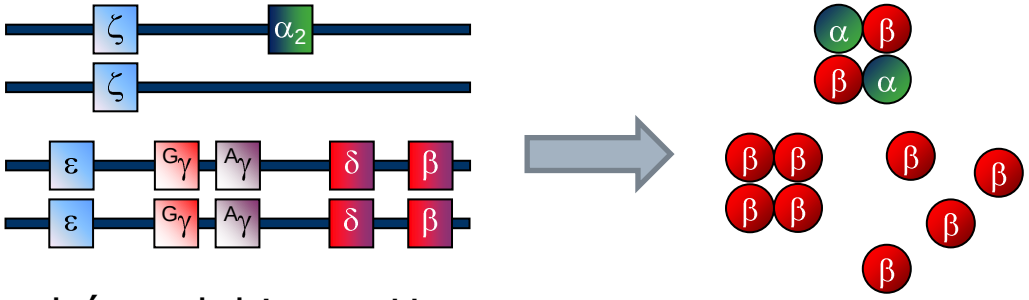
α-thalassémie 1



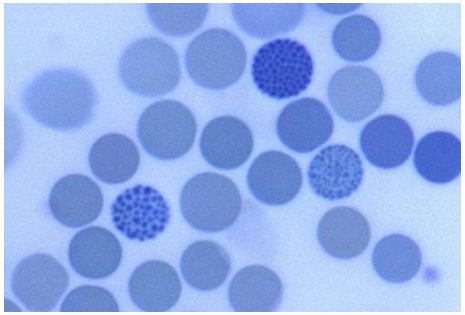
α-Thalassémie

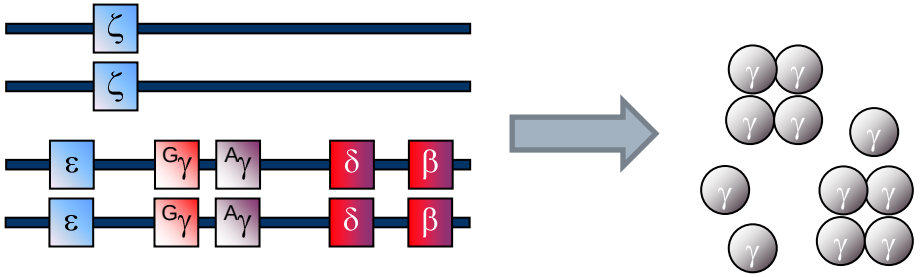
LHUB-ULB

α-thalassémies "majeures"

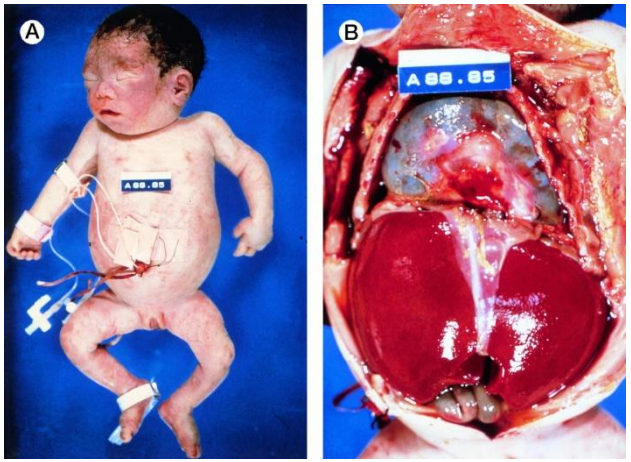


hémoglobinose H





Hb Bart's hydrops fetalis syndrome



Blood 1998;91:2213

Variants de l'hémoglobine

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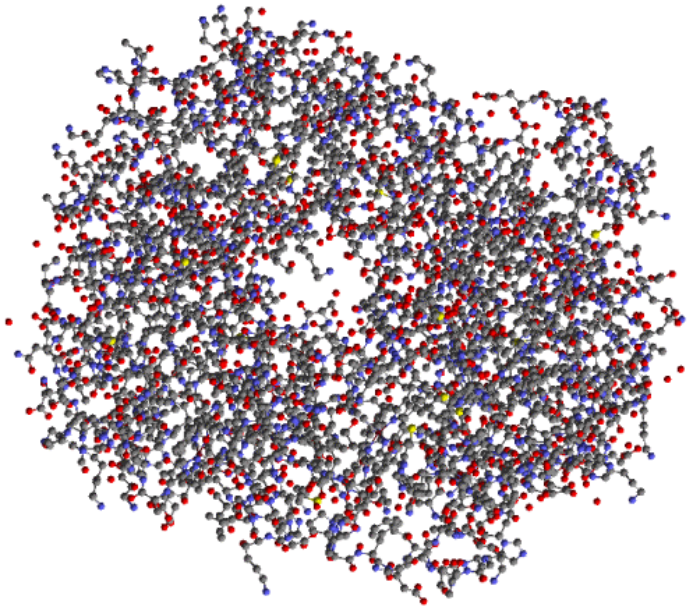
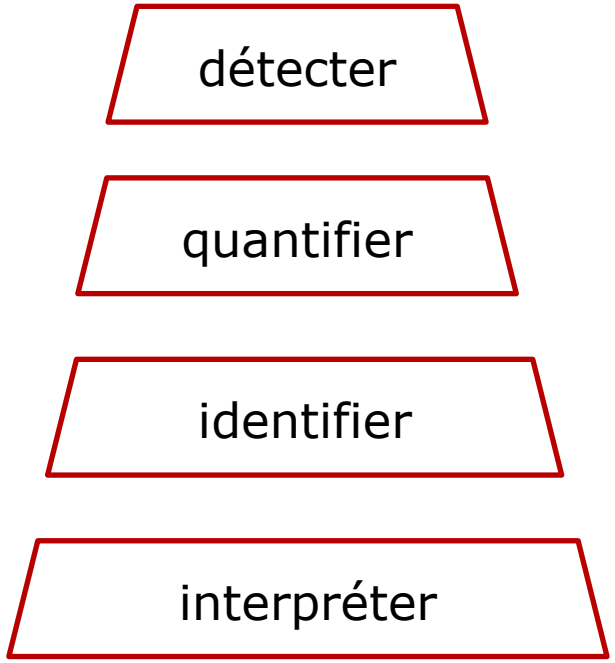
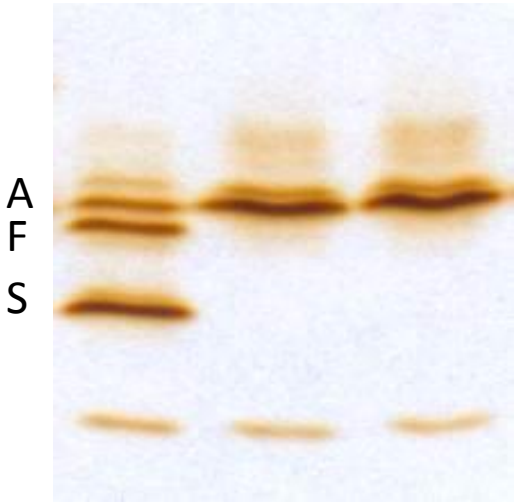
- mutations dans les zones codantes des gènes α et β
- 1247 variants connus
  - substitution
  - troncation
  - élongation
  - fusion
- 20% fonction anormale
  - polymérisation (S, C, D, E, O-Arab…)
  - affinité anormale
  - oxydation
  - instabilité
- Bruxelles: 2,3%



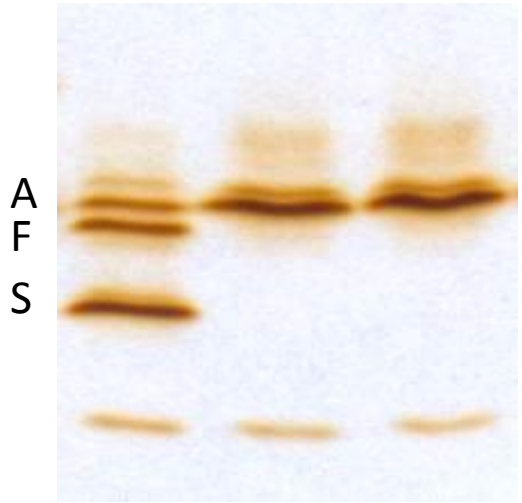
Démarche diagnostique

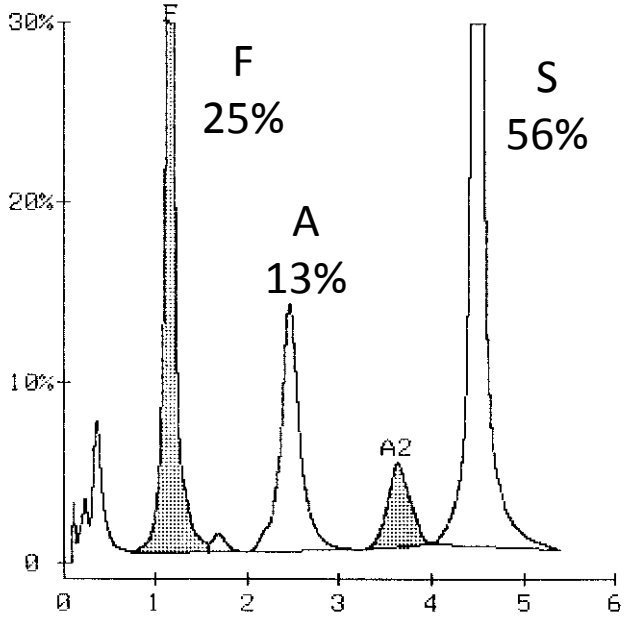


Démarche diagnostique



Démarche diagnostique





Analyse de l'hémoglobine

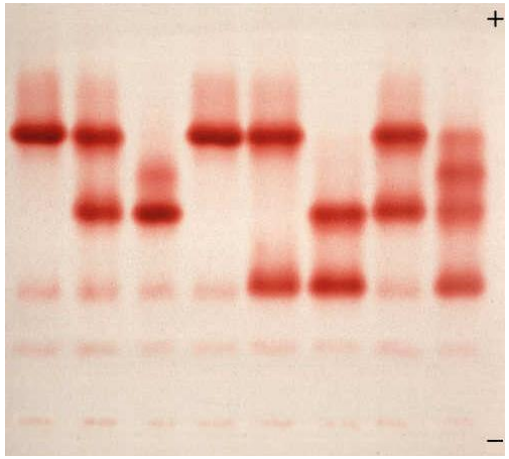
- tests de première ligne (détecter)
  - charge
  - fonctionnels
    - polymérisation
    - stabilité
    - affinité
    - oxydation

  variants courants
  et cliniquement significatifs
  [tout laboratoire]

- tests de seconde ligne et spécialisés (identifier)
  - charge
  - chaînes de globine
  - peptide
  - spectrométrie de masse
  - génétique

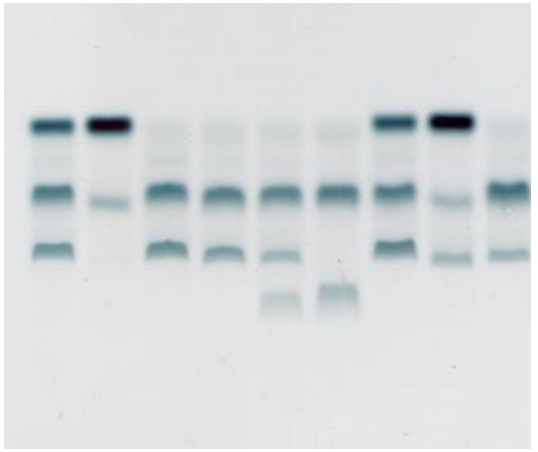
  variants courants
  et cliniquement significatifs
  [laboratoire spécialisé]

Electrophorèse



A
F
S, D, G
C, E, O, A_2

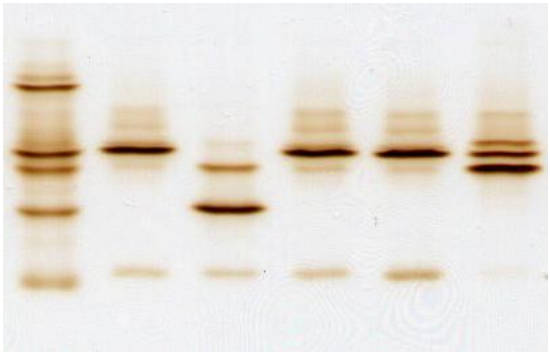
agarose/acétate cellulose pH 8.4



F
A, D, E, G, A_2
O
S
C

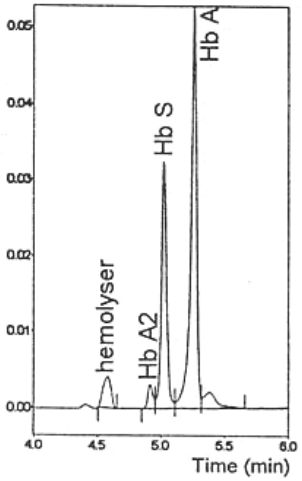
citrate agar/agarose pH 6.2

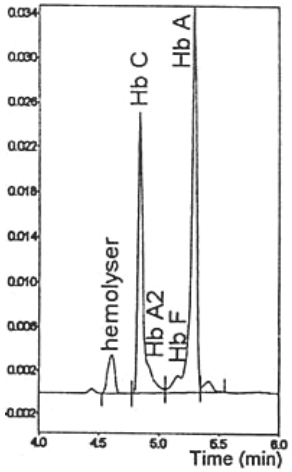
Hb	pI
N	6.80
A	6.98
F	7.05
S	7.21
A_2	7.40
C	7.42

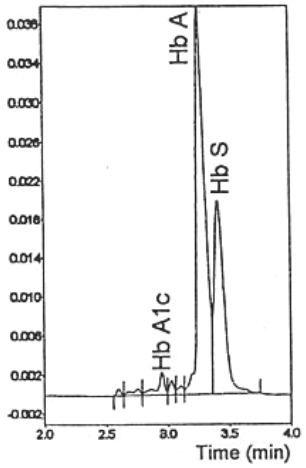


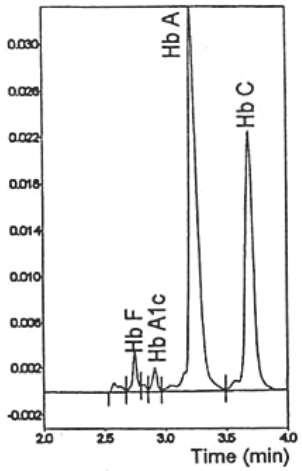
focalisation isolélectrique

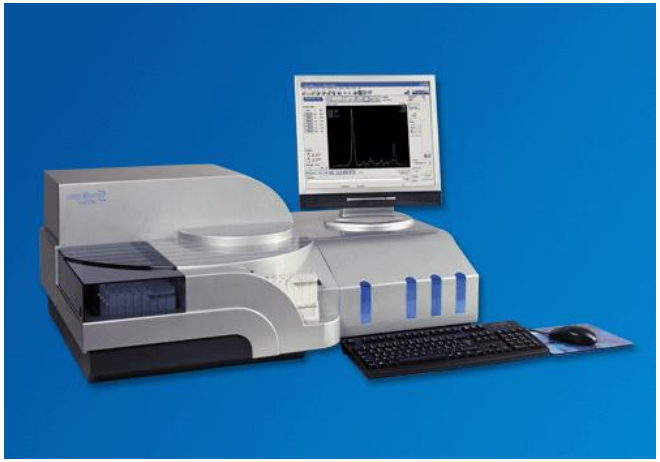
Electrophorèse capillaire





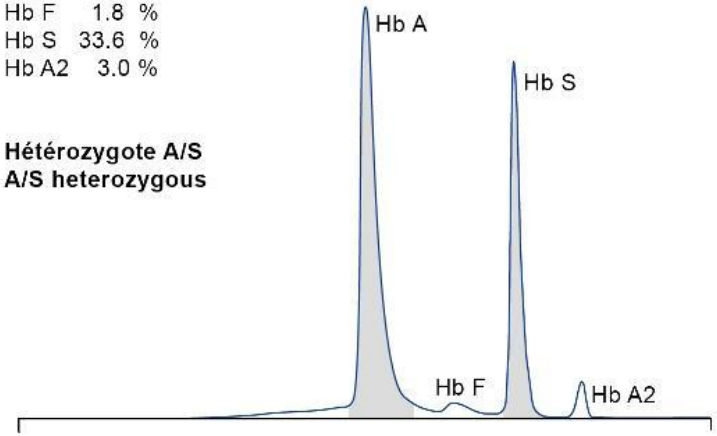




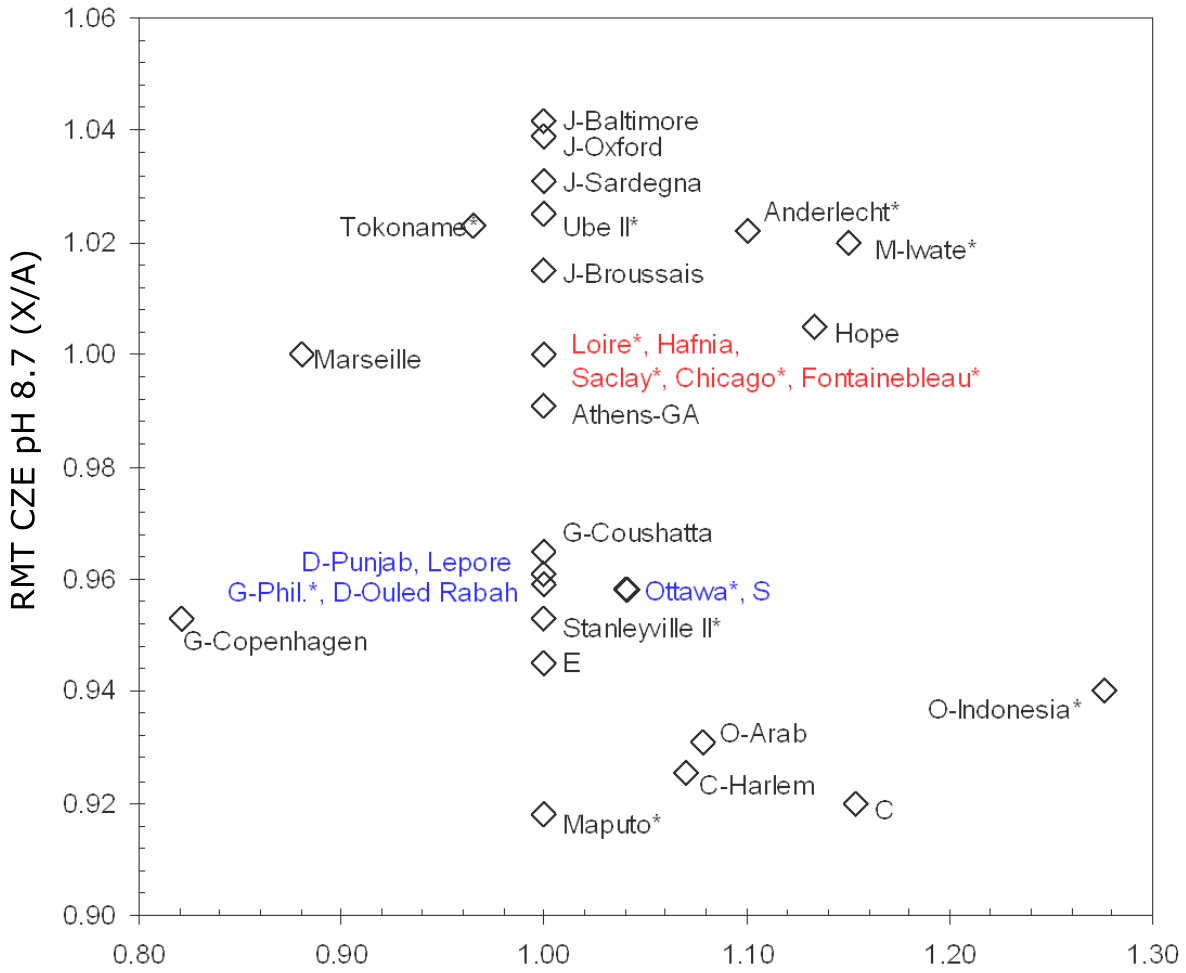


Hb A 61.6 %
Hb F 1.8 %
Hb S 33.6 %
Hb A2 3.0 %

Hétérozygote A/S
A/S heterozygous

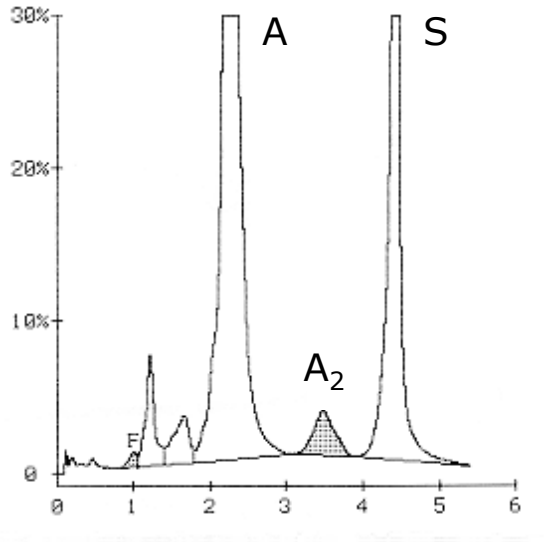


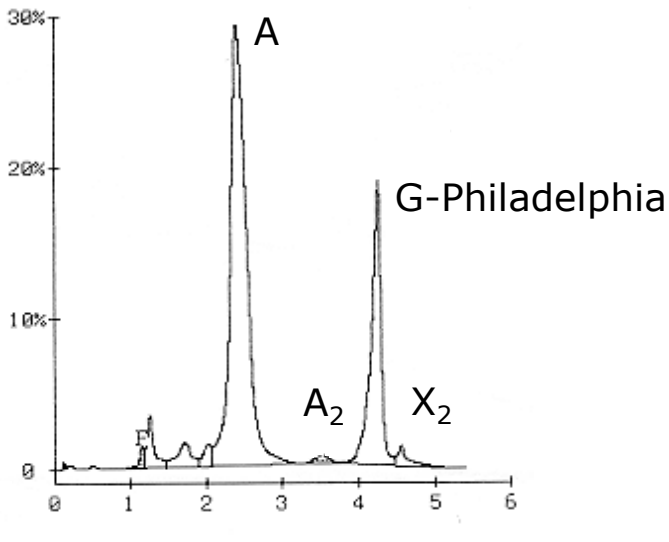
Electrophorèse capillaire



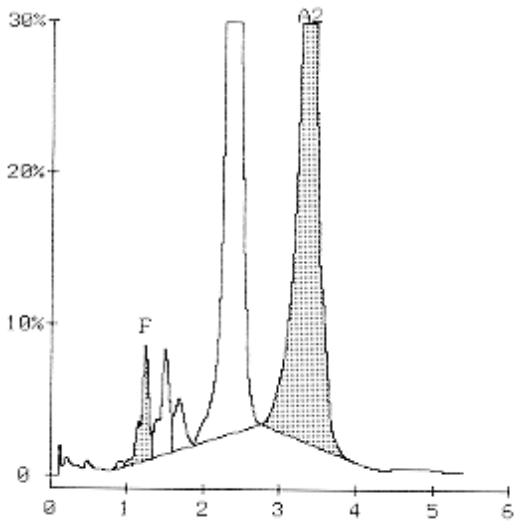
Ann Clin Biochem 2003;40:659

Chromatographie liquide

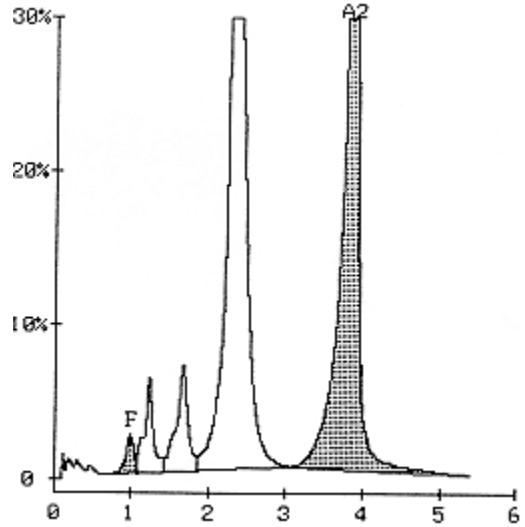




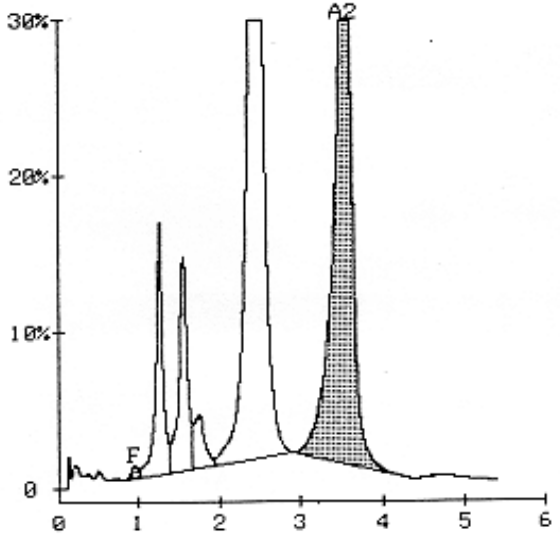
Chromatographie liquide



Hb G-Coushatta



Hb Korle-Bu

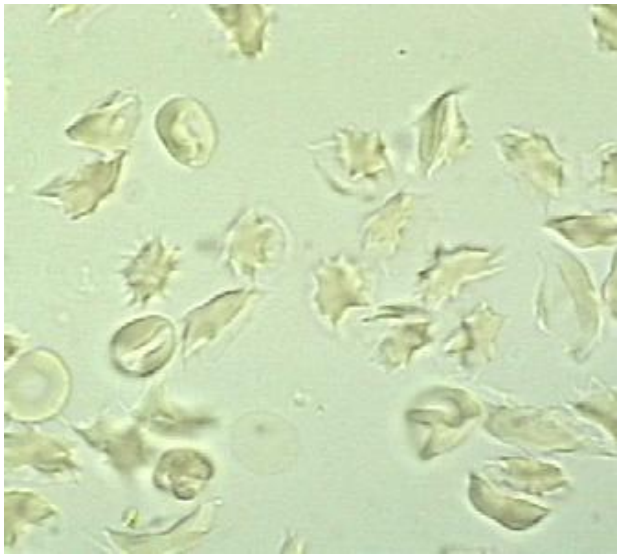


Hb D-Iran

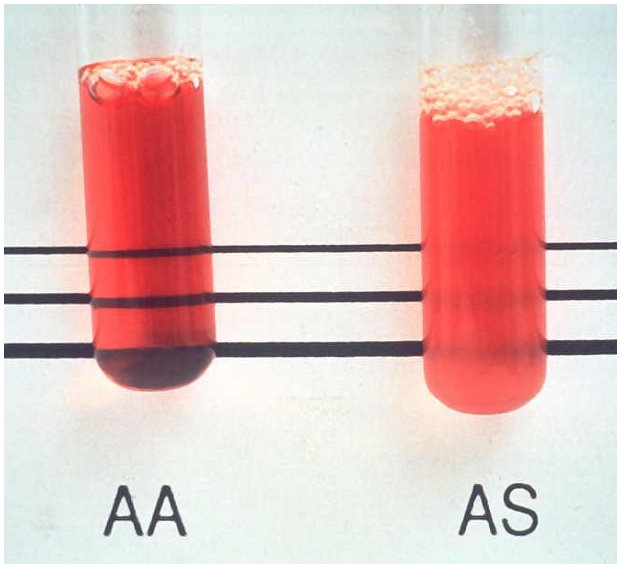
Analyse de l'hémoglobine

- tests de première ligne (détecter)
  - charge
  - **fonctionnels**
    - polymérisation
    - stabilité
    - affinité
    - oxydation
- tests de seconde ligne et spécialisés (identifier)
  - charge
  - chaînes de globine
  - peptide
  - spectrométrie de masse
  - génétique

Polymérisation

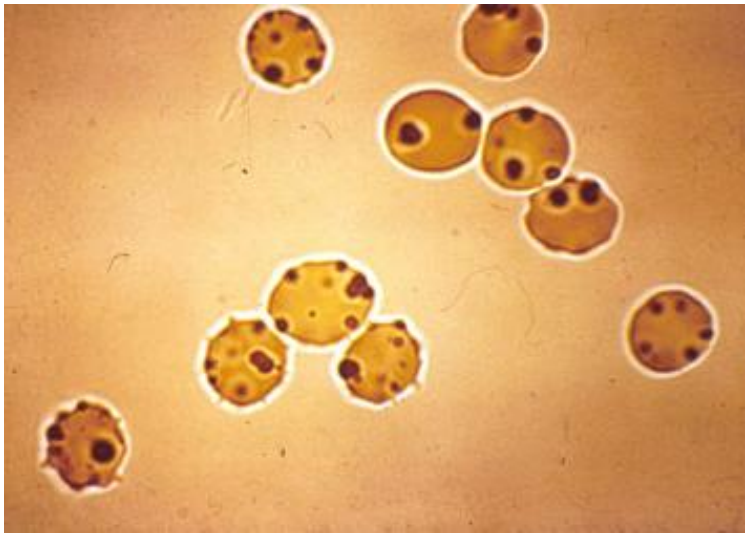


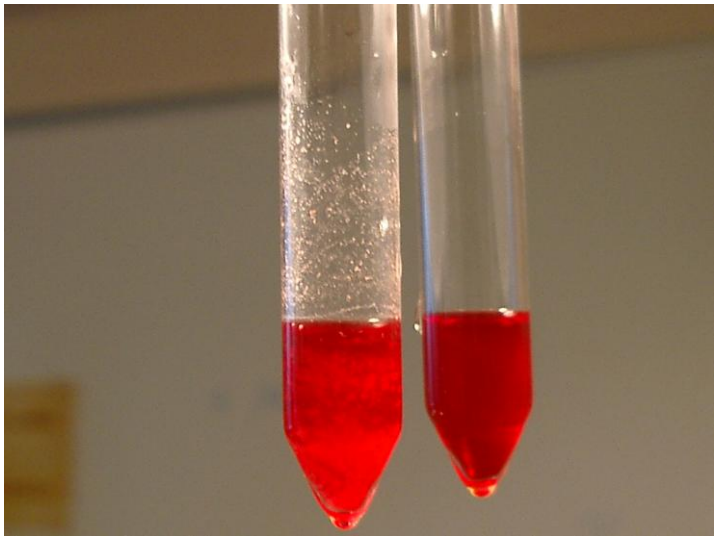
Test d'Emmel



Test d'Itano (solubilité)

Instabilité



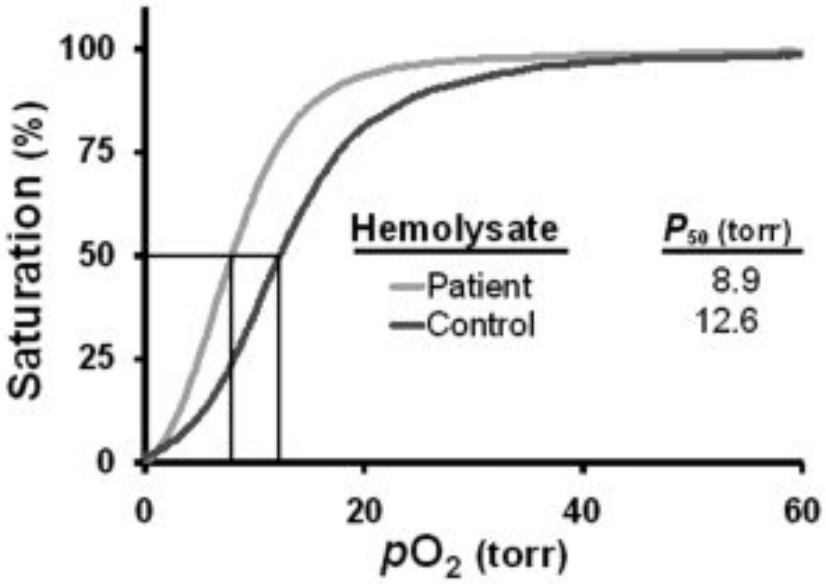


Test à l'isopropanol
(à la chaleur)

Hemoglobin 1990;14:137

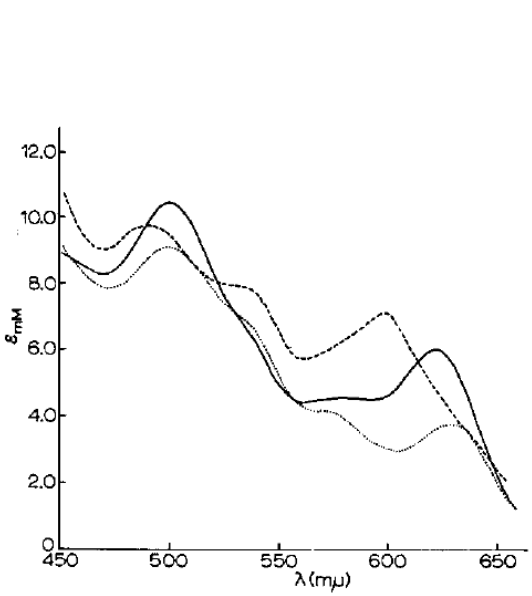
Affinité

- hypoglobulie
- polyglobulie
  - récepteur EPO
  - régulation EPO
  - déf. 2,3 BPG mutase



Am J Hematol 2010;85:848

Oxydation



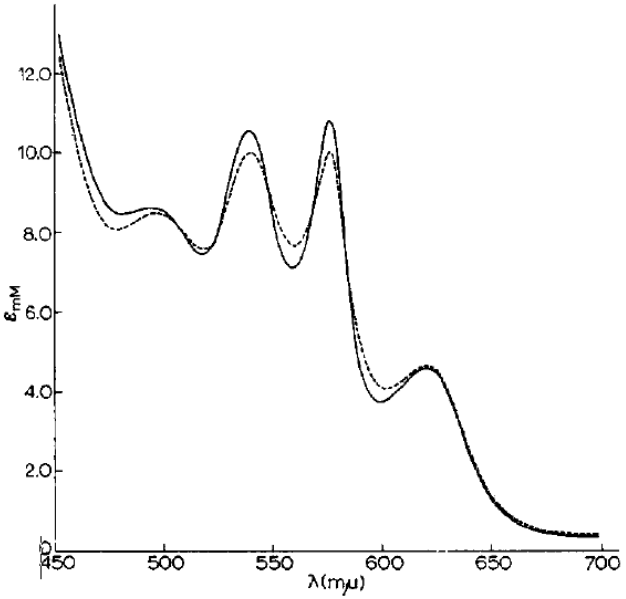


Fig. 2. Visible spectra of MetHb M Milwaukee-1, MetHb M Saskatoon and MetHb A in 0.1 M potassium phosphate buffer (pH 7.0). ———, MetHb M Milwaukee-1; — — —, MetHb M Saskatoon; · · · · ·, MetHb A.

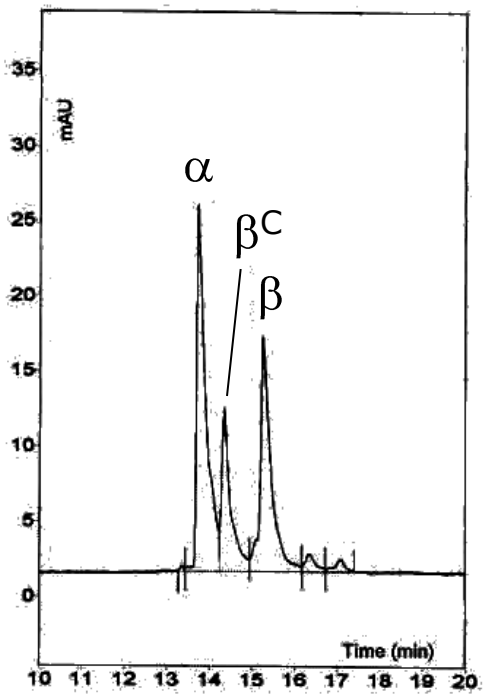
Fig. 3. Visible spectra of HbO_2 M Milwaukee-1 in 0.1 M potassium phosphate buffer (pH 6.5) under air and under pure O_2 gas. The spectra at pH 7.9 under air or pure O_2 gas were essentially the same with the spectra at pH 6.5 under pure O_2 gas. — — —, HbO_2 M Milwaukee-1 at pH 6.5 under air; ———, HbO_2 M Milwaukee-1 at pH 6.5 under pure O_2 gas.

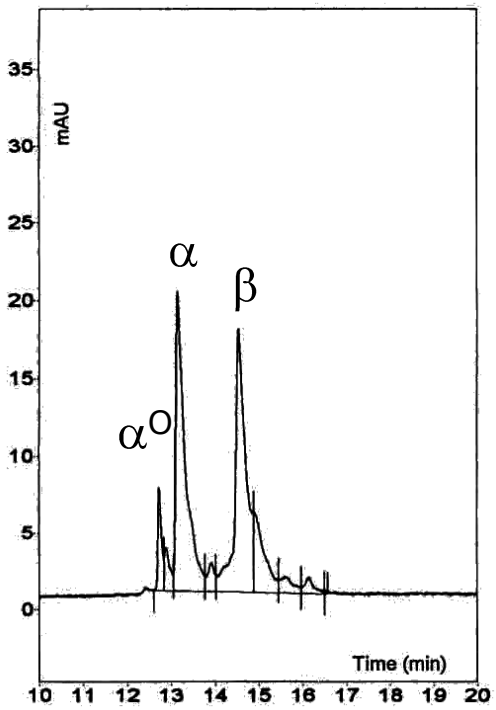
Biochim Biophys Acta 1969;194:6

Analyse de l'hémoglobine

- tests de première ligne (détecter)
  - charge
  - fonctionnels
    - polymérisation
    - stabilité
    - affinité
    - oxydation
- **tests de seconde ligne et spécialisés (identifier)**
  - charge
  - chaînes de globine
  - peptide
  - spectrométrie de masse
  - génétique

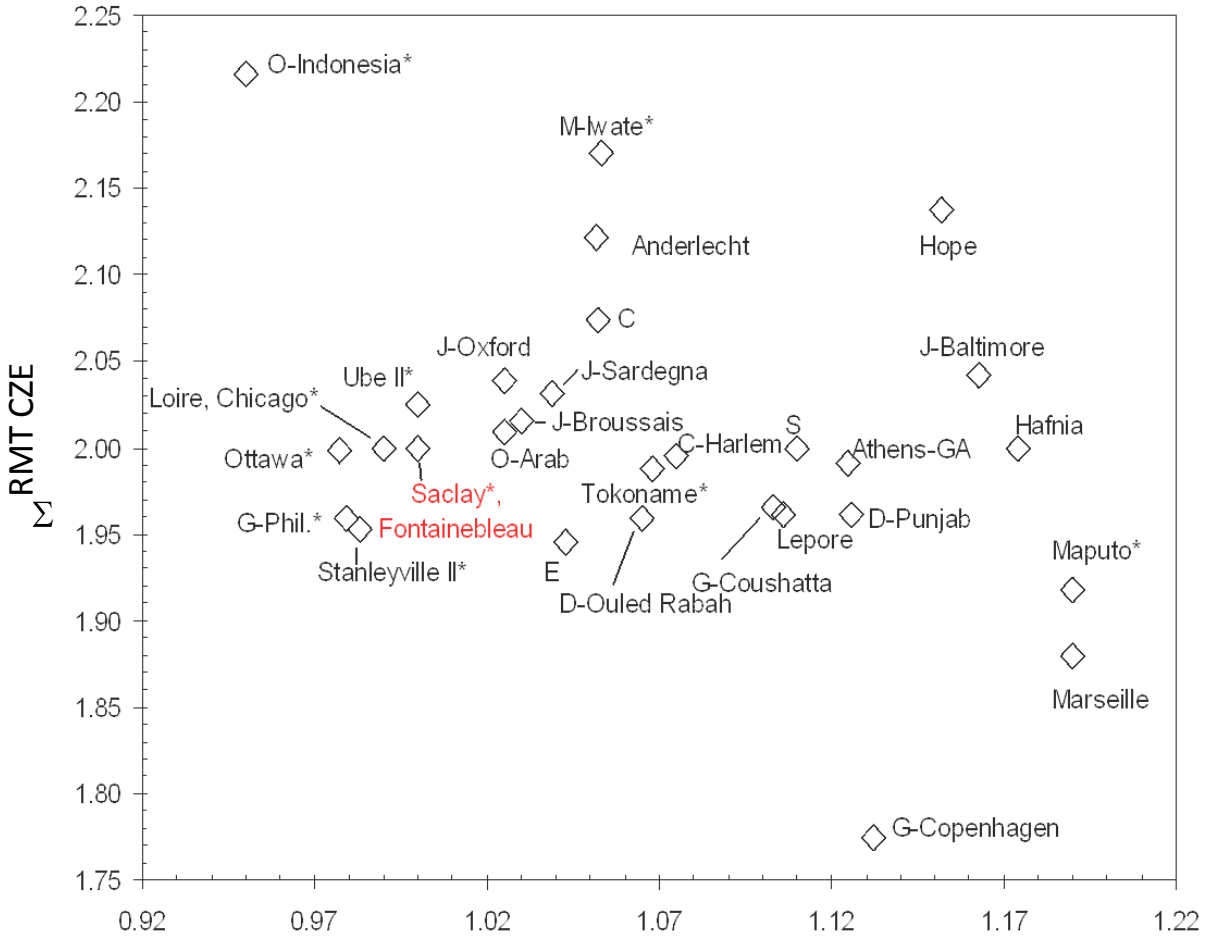
Chaînes de globine





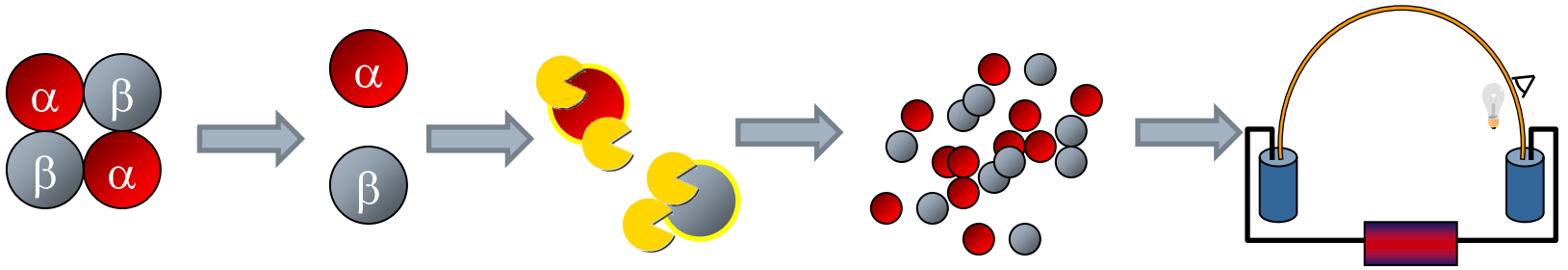
J Chromatogr B 1998;719:47

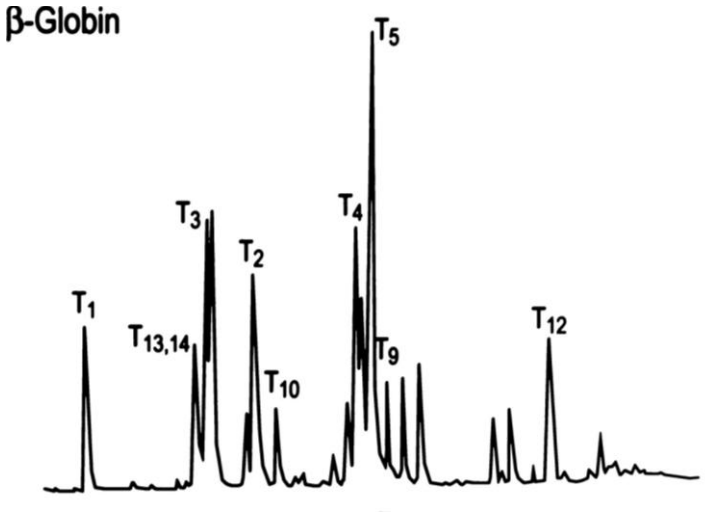
Chaînes de globine

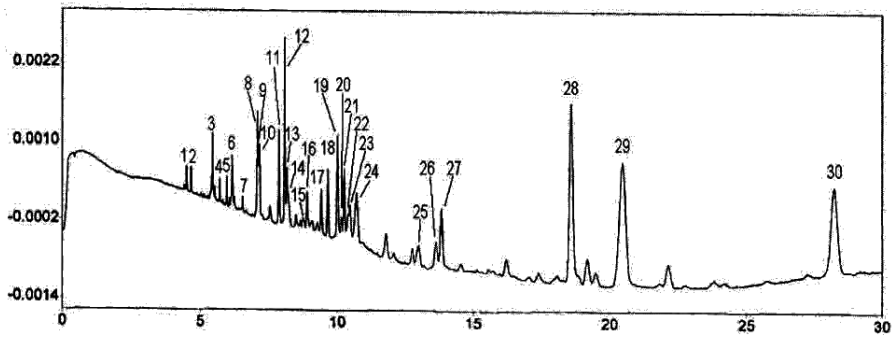


Ann Clin Biochem 2003;40:659

Cartographie peptidique

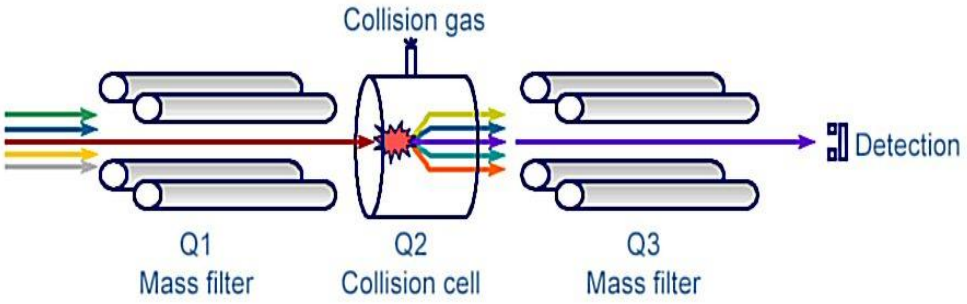


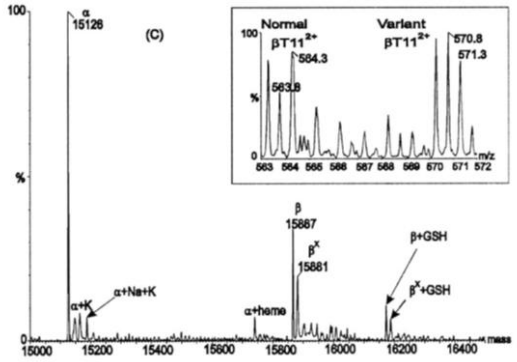


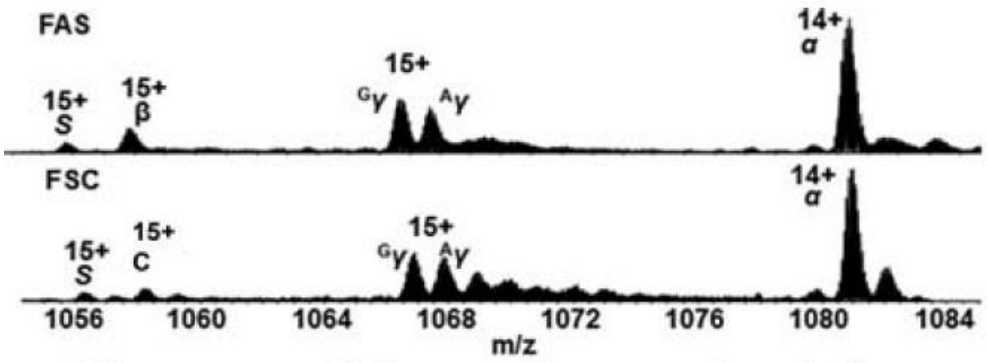


Hemoglobin 2001;25:259

Spectrométrie de masse



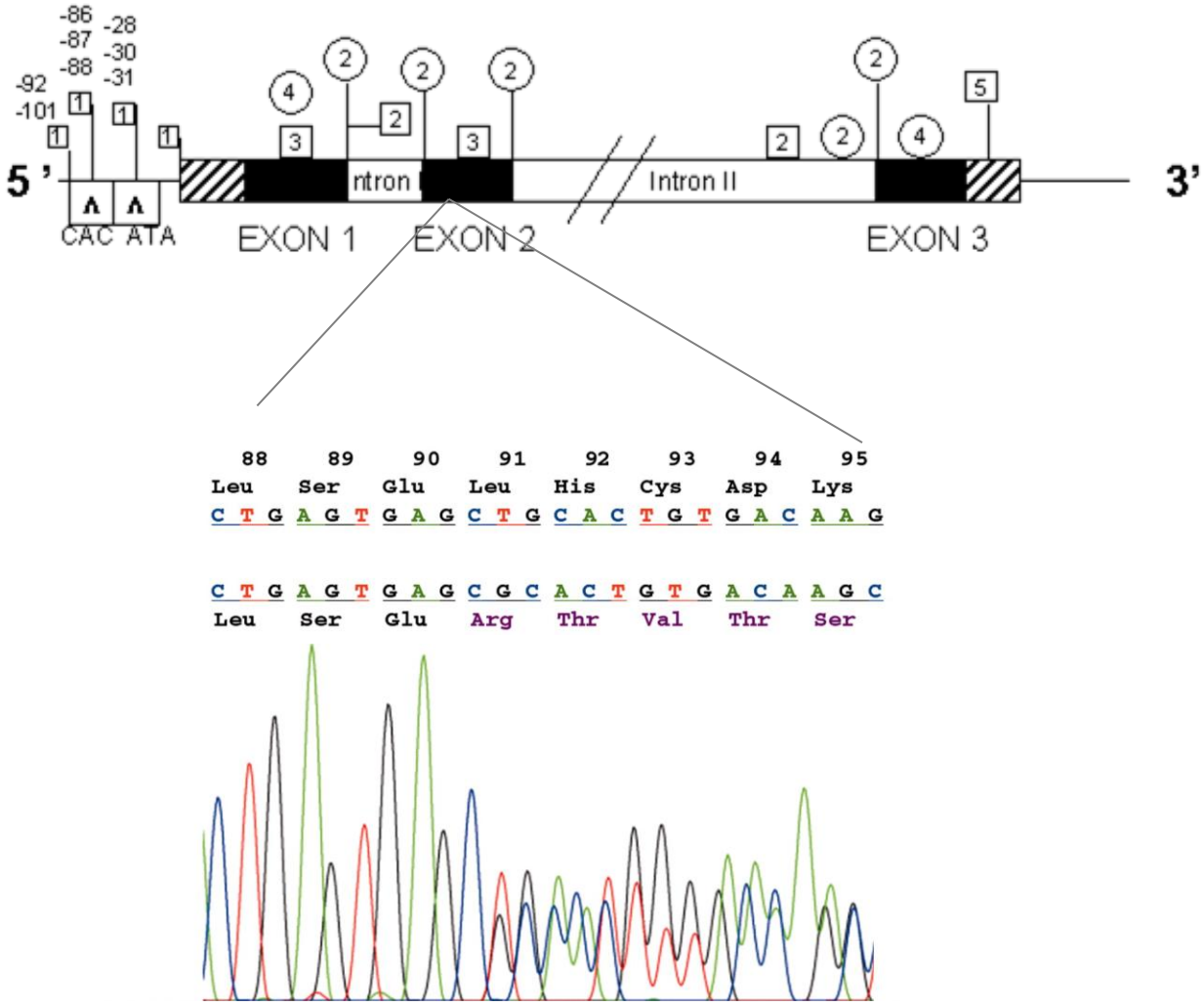




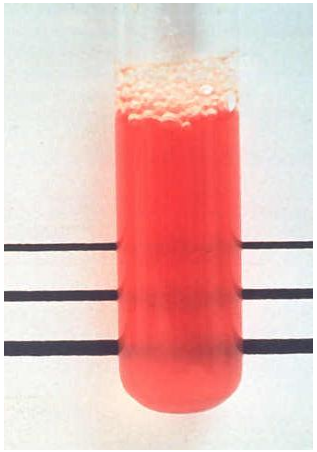
Clin Chem 2001;47:1308

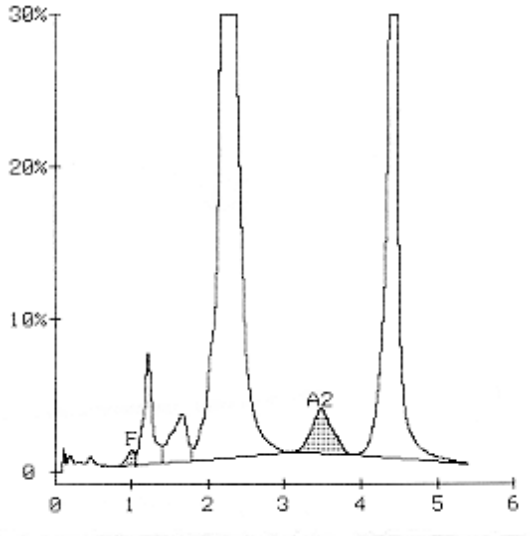
Proteomics 2014;14:1232

Séquençage


-86
-87
-88
-92
-101
-28
-30
-31
5 '
3'
CAC ATA
EXON 1
EXON 2
EXON 3
Intron II
88 89 90 91 92 93 94 95
Leu Ser Glu Leu His Cys Asp Lys
C T G A G T G A G C T G C A C T G T G A C A A G
C T G A G T G A G C G C A C T G T G A C A A G C
Leu Ser Glu Arg Thr Val Thr Ser

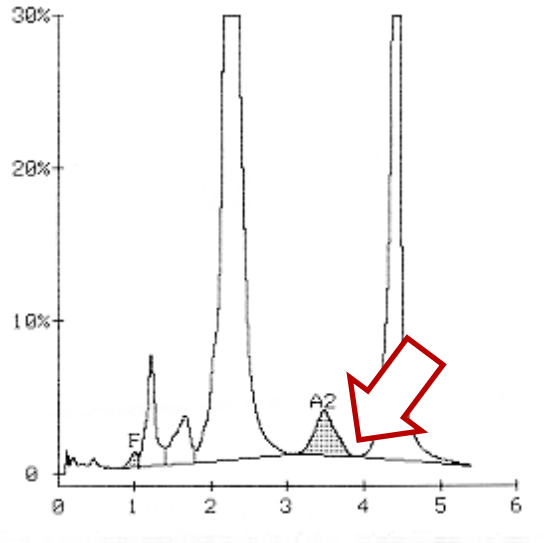
Interprétation





Hb F	0.6%
P2	2.1%
P3	1.2%
A	58%
A2	4.1%
S	34%

Interprétation



Recherche d'hémoglobinopathie

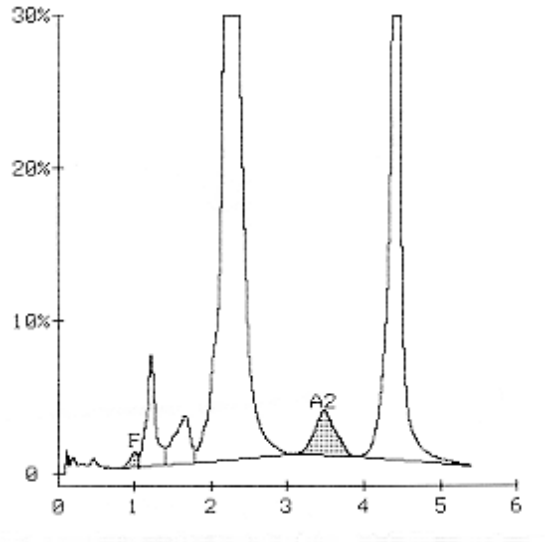
Hb A + "Hb S" (34%)

Hb F 0.6%

~~Hb A_2 4.1%~~

Hb F	0.6%
P2	2.1%
P3	1.2%
A	58%
A2	4.1%
S	34%

Interprétation

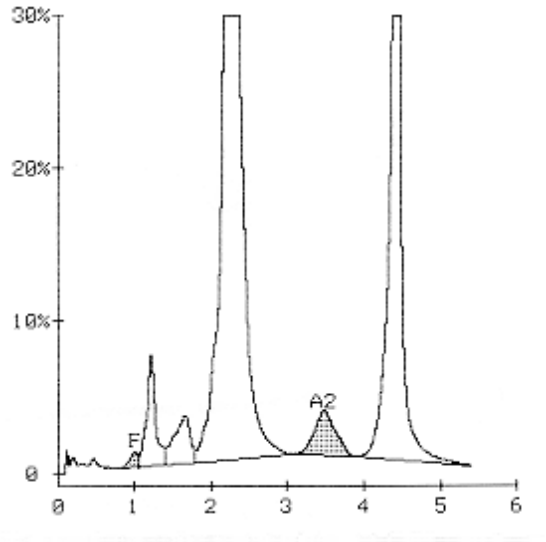


Recherche d'hémoglobinopathie
Hb A + Hb S probable (34%)
Hb F 0.6%

Conclusion
Hétérozygote S probable

Hb F	0.6%
P2	2.1%
P3	1.2%
A	58%
A2	4.1%
S	34%

Interprétation

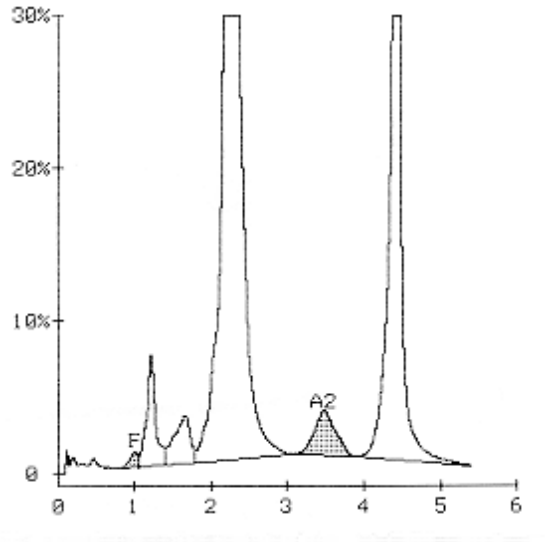


Hb F	0.6%
P2	2.1%
P3	1.2%
A	58%
A2	4.1%
S	34%

Recherche d'hémoglobinopathie
Hb A + Hb S probable (34%)
Hb F 0.6%

Conclusion
Hétérozygote S probable
-taux Hb S bas
-carence fer
-α-thalassémie } MCV, MCH

Interprétation



Hb F	0.6%
P2	2.1%
P3	1.2%
A	58%
A2	4.1%
S	34%

Recherche d'hémoglobinopathie

Hb A + Hb S probable (34%)

Hb F 0.6%

Conclusion

Hétérozygote S probable

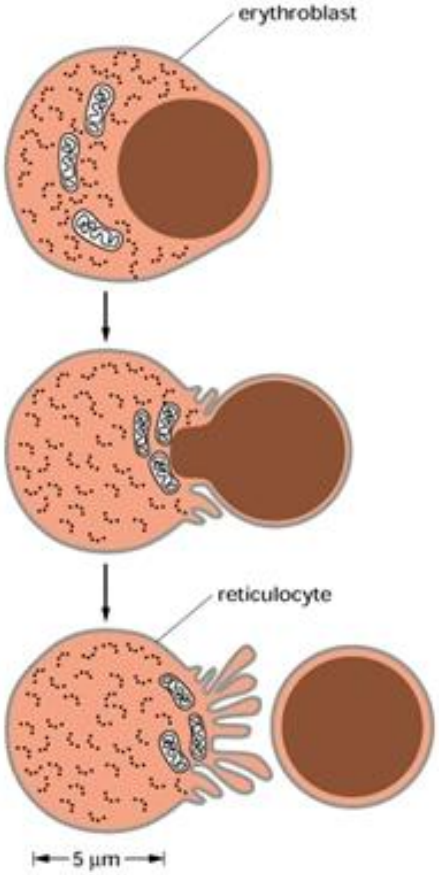
- -taux Hb S bas
  - -carence fer
  - -α-thalassémie
- -test du partenaire
- -conseil génétique

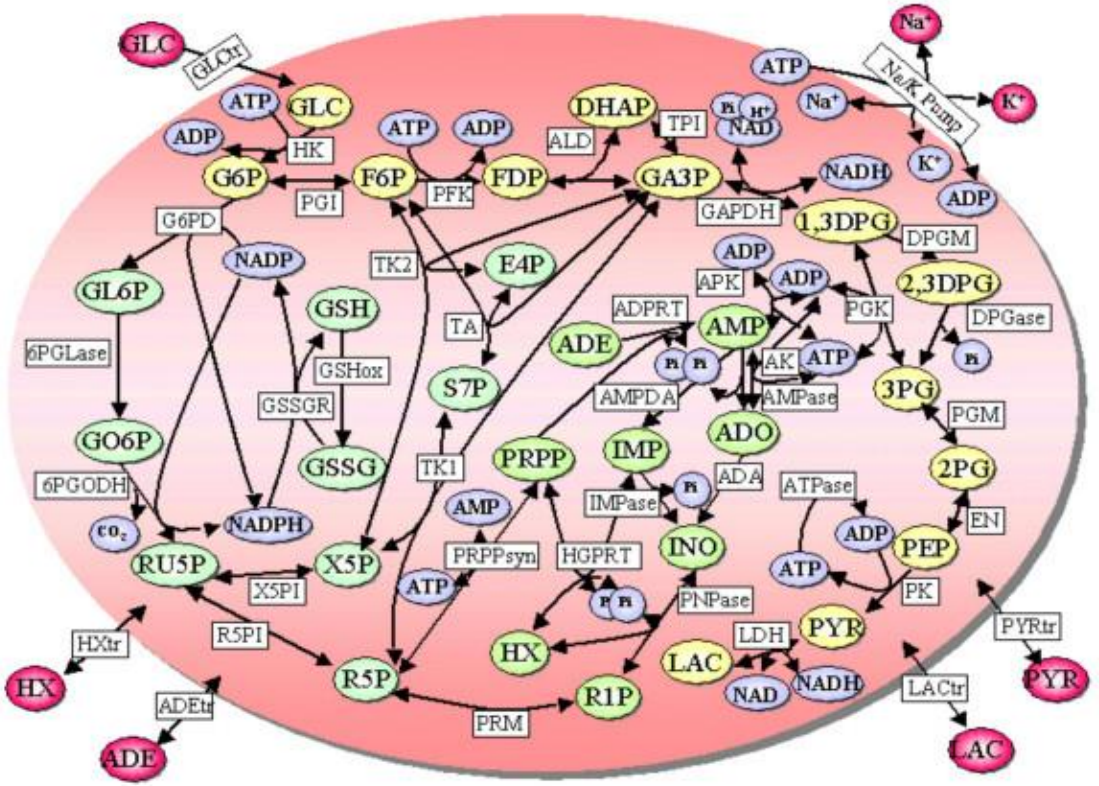
Interprétation

Plan

1. Hémolyse
2. Hémoglobinopathies
3. **Enzymopathies érythrocytaires**
4. Membranopathies

Métabolisme érythrocytaire

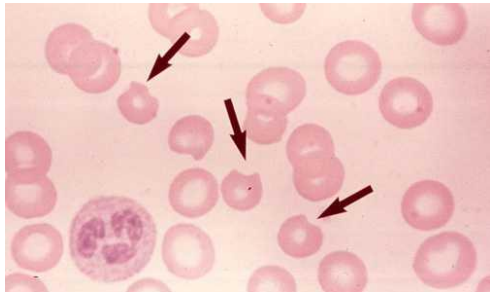


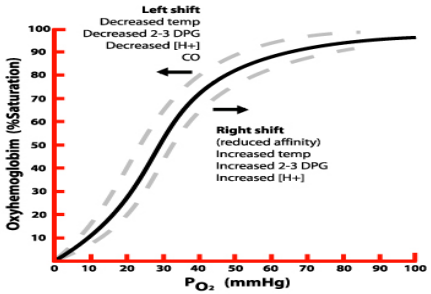


Theor Biol Med Model 2005;2:18

Déficits enzymatiques

- anémie hémolytique
  - aiguë
  - chronique (+…)
- affinité Hb augmentée
- oxydation Hb



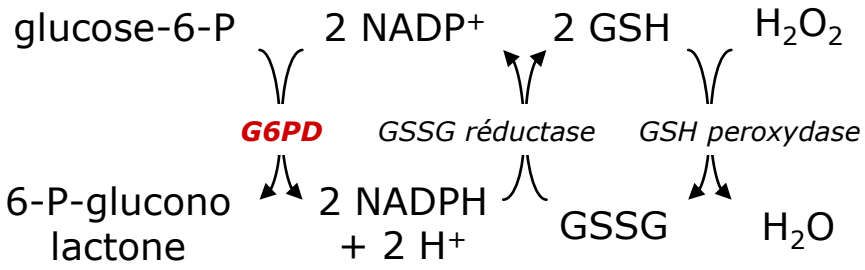


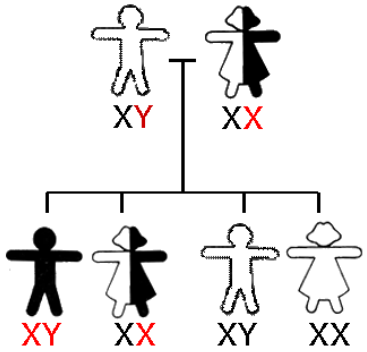
Déficits enzymatiques

Enzyme	Prévalence	Hémolyse aiguë	Hémolyse chronique	Autres atteintes
G6PD	+++	X	(X)	
PK	++		X	
GPI	+		X	(X)
P5N	+		X	
ADA (↑)	+		X	
EN	(+)		X	
PFK	(+)		X	(X)
ALD	(+)		X	(X)
GS, GR, GPX	(+)	X	X	(X)
TPI	+		X	X
PGK	(+)		X	X
HK	(+)		X	

G6PD

- production de NADPH
  - métabolisme du glutathion
  - protection contre l'oxydation
- déficience
  - prévalence mondiale 4,9%
  - liée à X

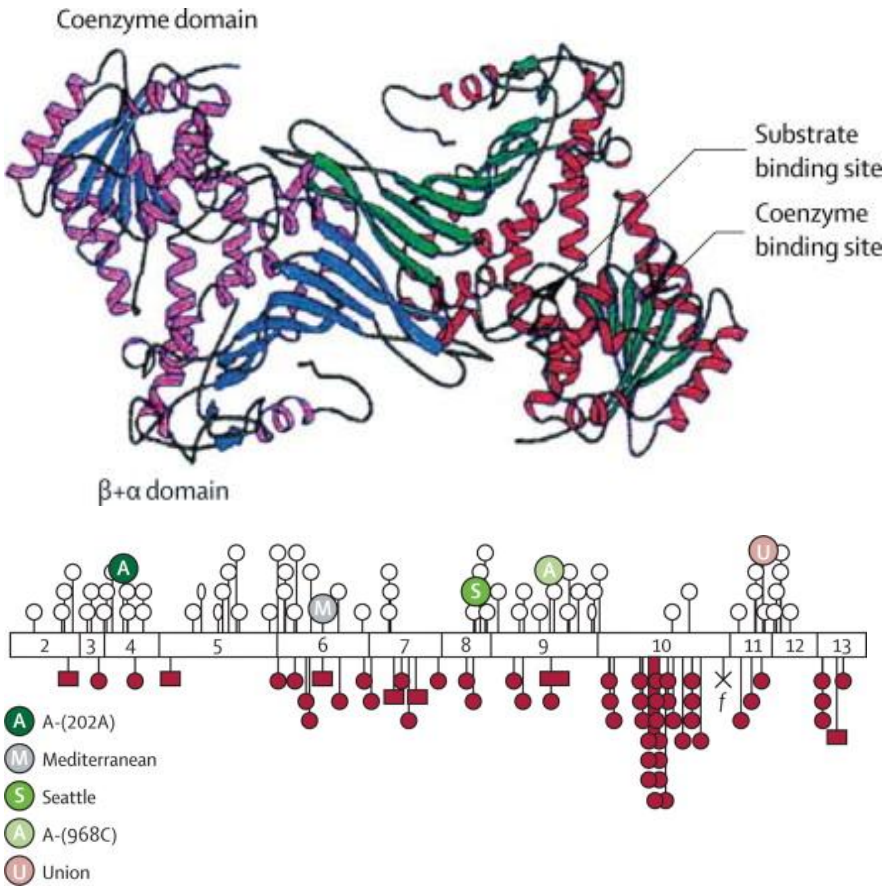




Blood Cells Mol Dis 2009;42:267

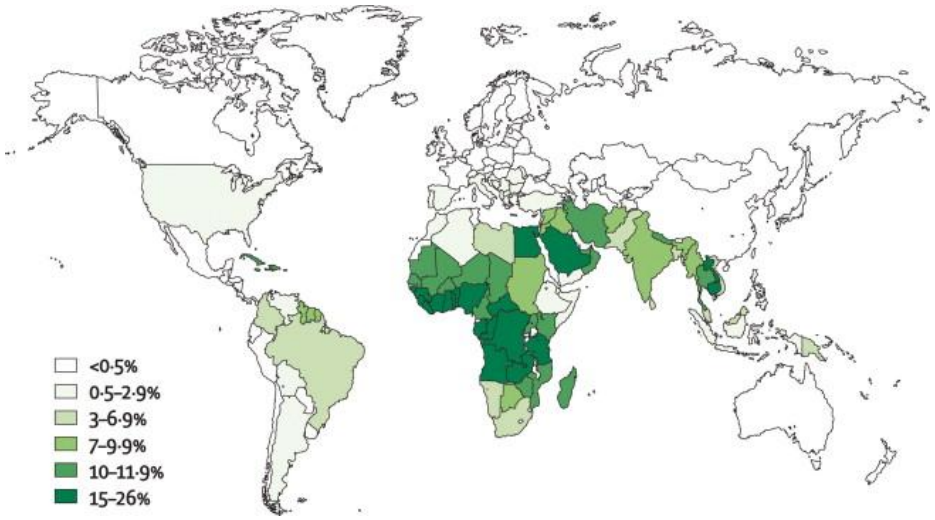
G6PD

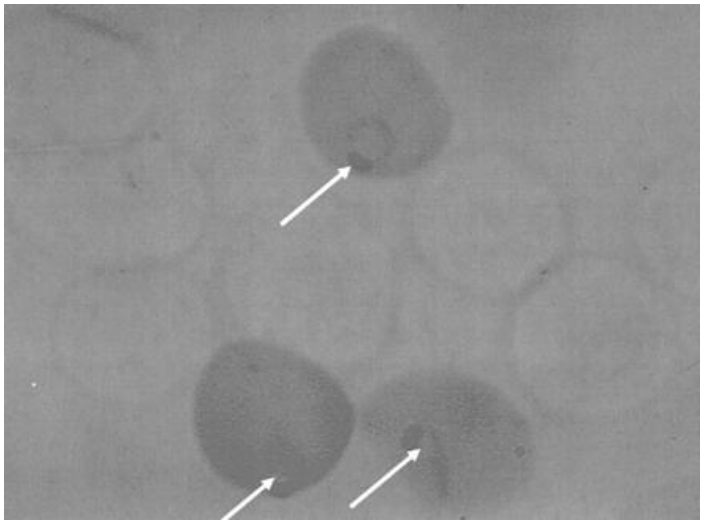
- 160 mutations
- classes
  - I: <1%
  - II: 1-10%
  - III: 10-60%
  - IV & V: >60%



Lancet 2008;371:64

G6PD

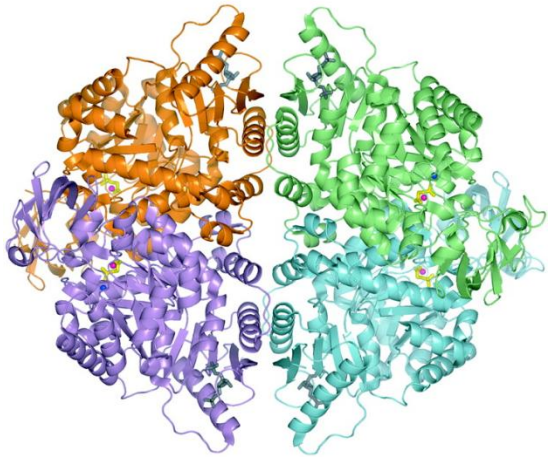


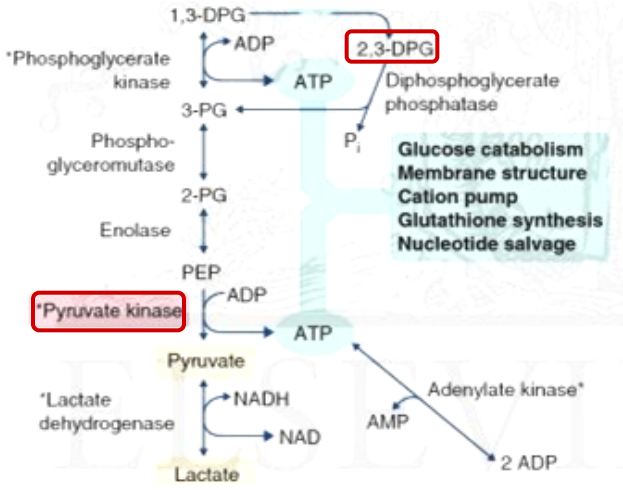


Blood 2008;111:16

Pyruvate kinase

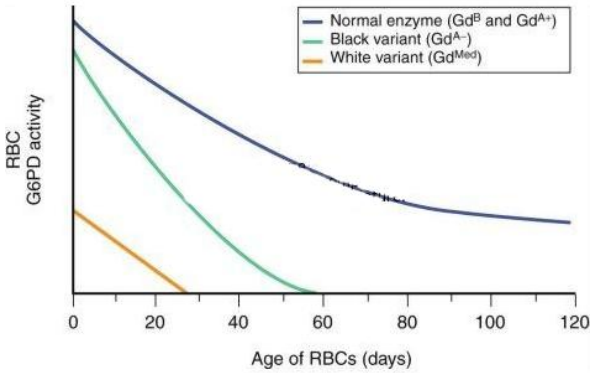
- production ATP, régulatrice
- 2 gènes, 4 isoenzymes
  - PK-LR (chromosome 1) [PK-L, PK-R]
  - PK M (chromosome 15) [PK-M1, PK-M2]
  - 190 mutations
    - prévalence: 1/20000
    - répartition mondiale, malaria ?

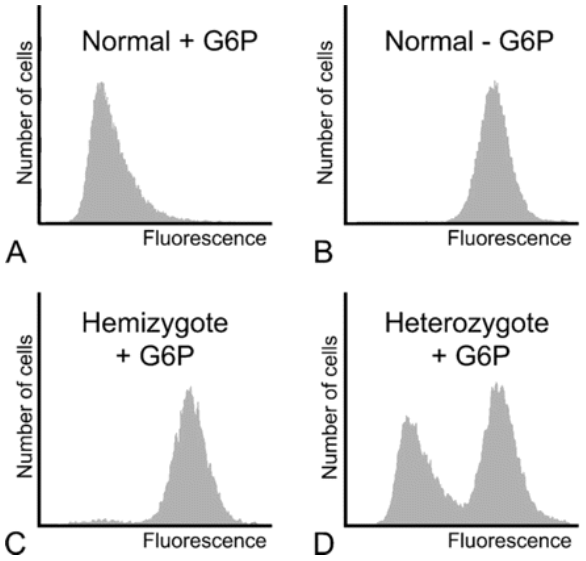




Diagnostic

- activité enzymatique
  - bonne corrélation clinique
  - U/g Hb – U/L RBC
  - pièges
    - réticulocytes
    - instabilité in vitro
- cytochimie
- biologie moléculaire
  - relation génotype-phénotype ?
  - anténatal
- cytométrie en flux

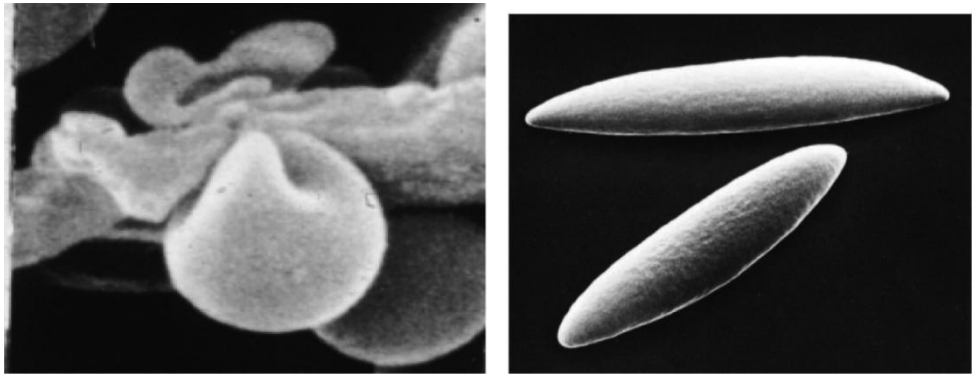


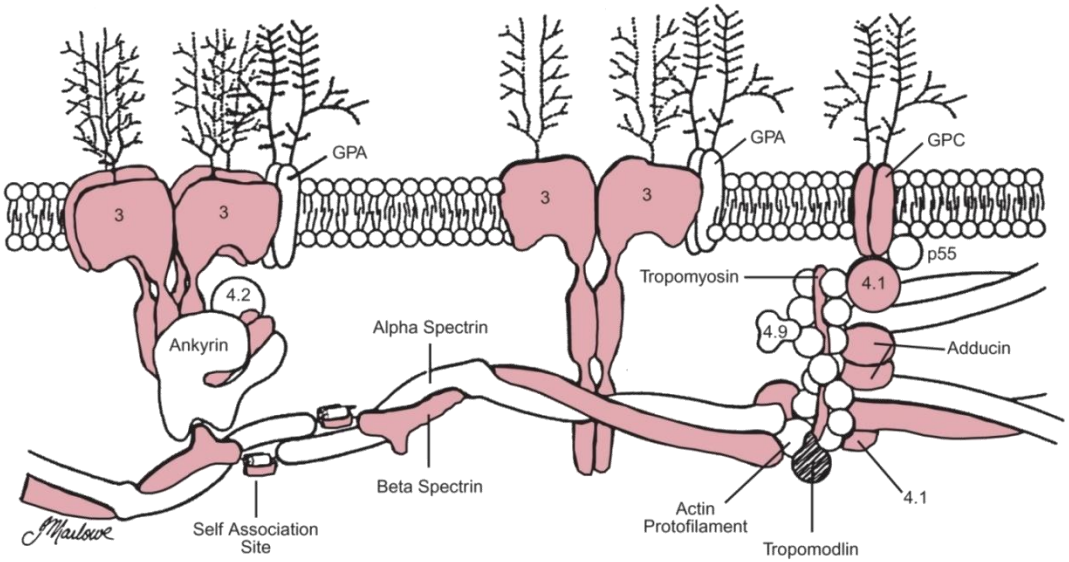


Plan

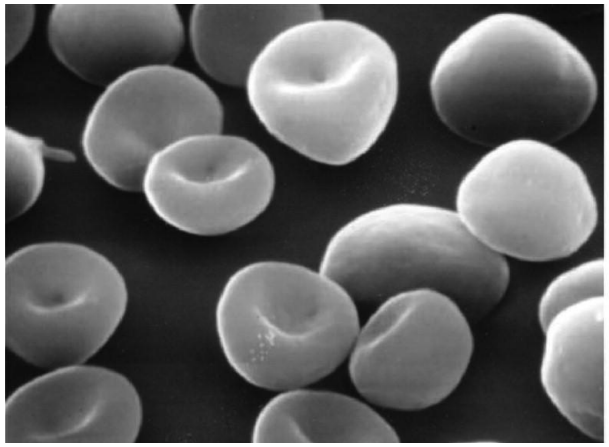
1. Hémolyse
2. Hémoglobinopathies
3. Enzymopathies érythrocytaires
4. **Membranopathies**

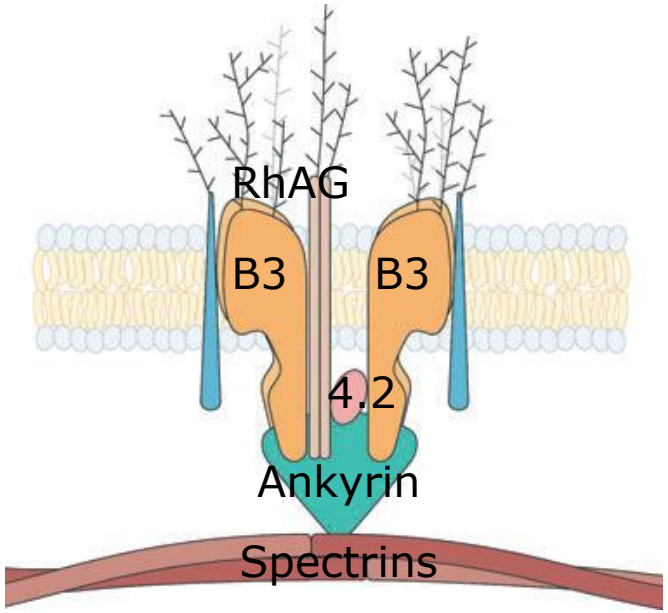
Membrane




GPA
GPA
GPC
3
3
3
3
p55
Tropomyosin
4.1
4.2
4.9
Ankyrin
Alpha Spectrin
Adducin
Beta Spectrin
4.1
Actin
Protofilament
Self Association
Site
Tropomodlin

Sphérocytose





< 1%

15-20%

< 5%

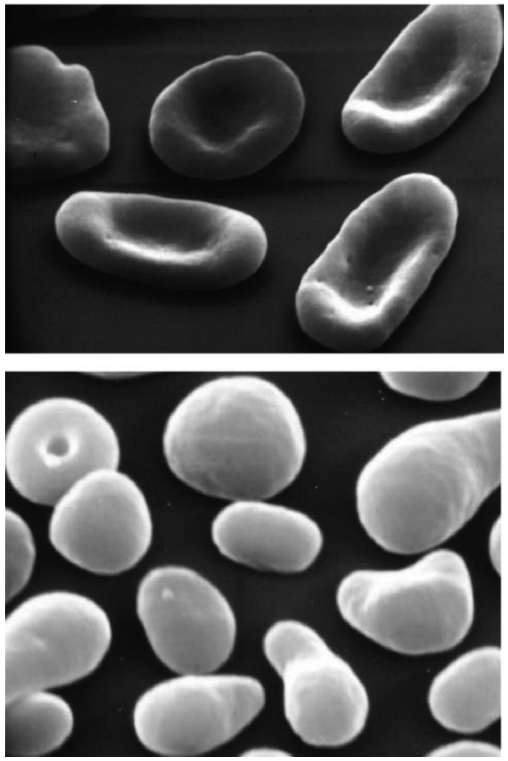
50-60%

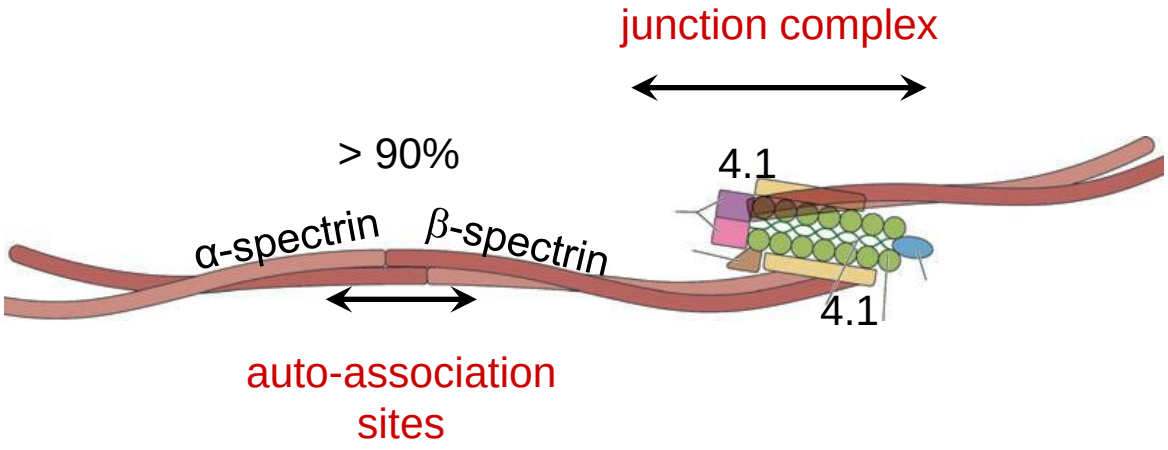
20%

- prévalence: 1/2000-1/5000
- transmission
  - AD (65%)
  - de novo (25%)
  - AR (10%)
- sévérité variable

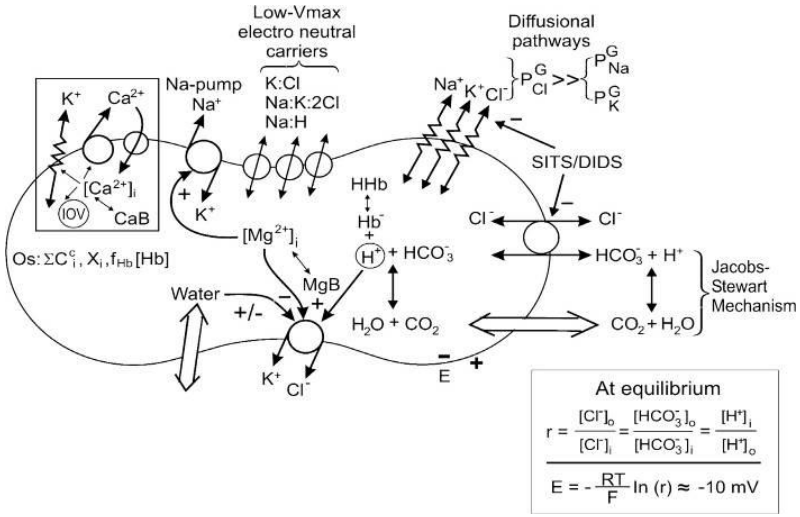
Br J Haematol 2008;141:367-75

Elliptocytose

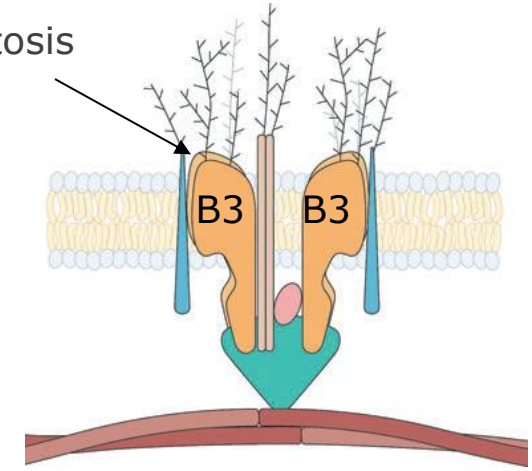




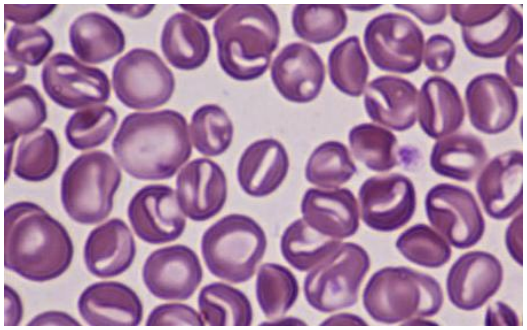
Stomatocytose



cryohydrocytosis

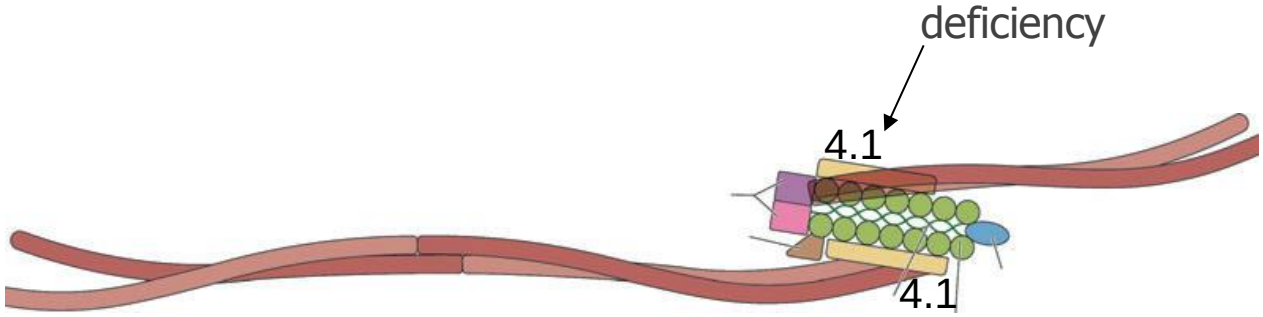


- dehydrated form (DHSt) (xerocytose)
- overhydrated form (OHSt) (hydrocytosis)
- intermediate forms
  - cryohydrocytosis
  - familial pseudohyperkaliemia

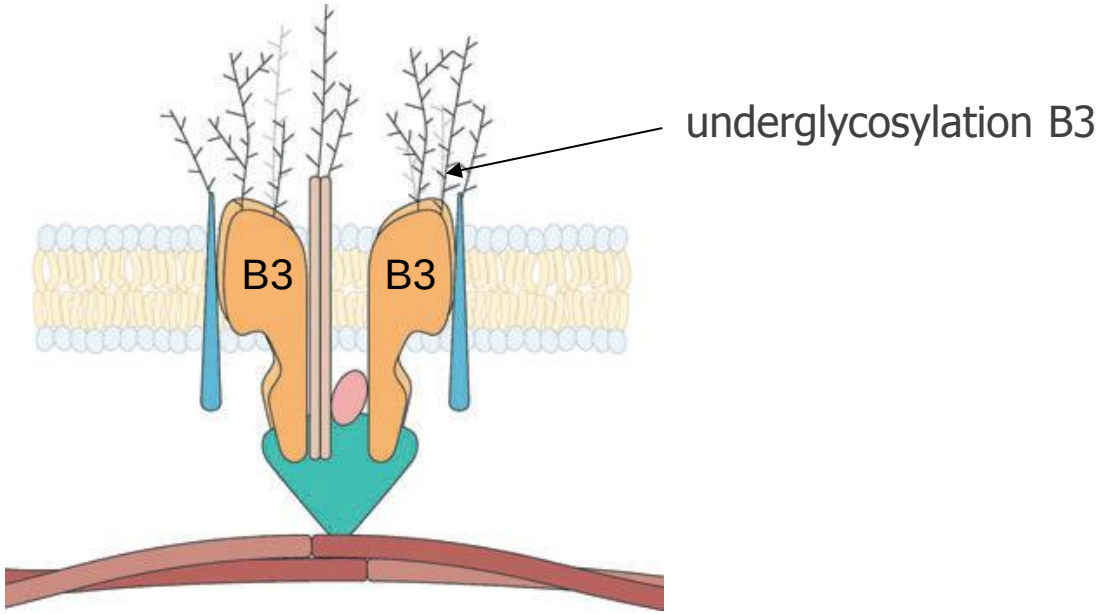


CDA

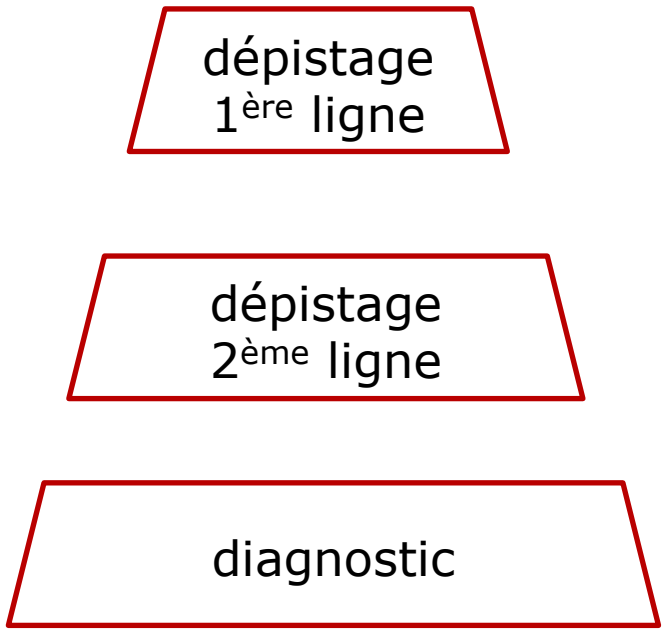
CDA I



CDA II



Démarche diagnostique

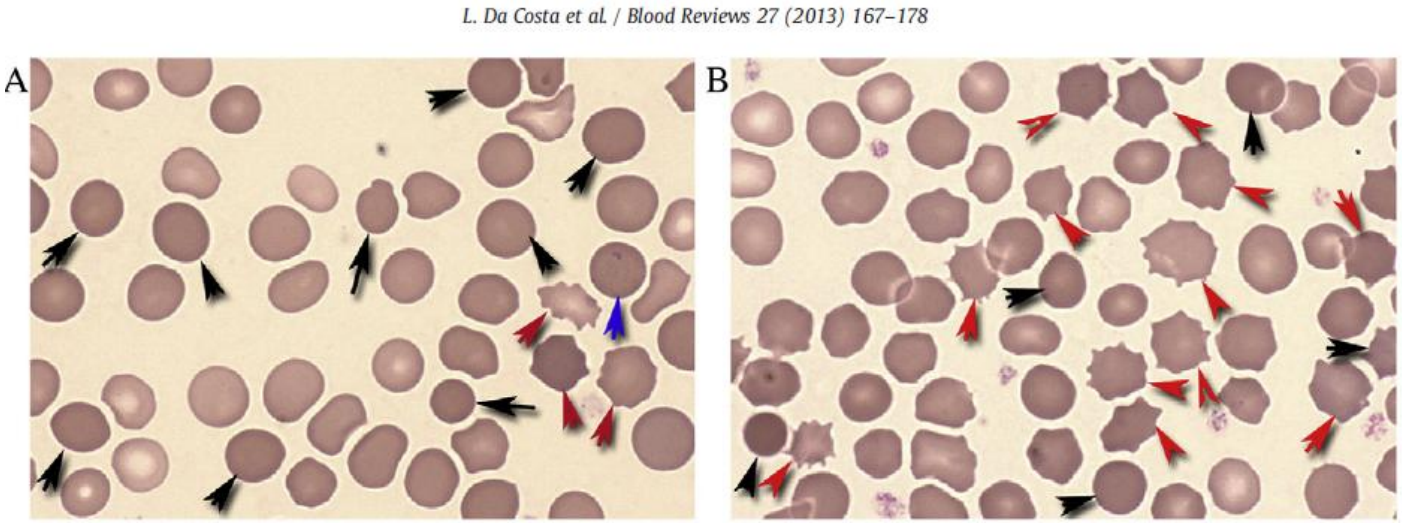


- morphologie
- indices érythrocytaires
- réticulocytes

- cryohémolyse hypertonique
- éosine-5-maléimide
- (résistance osmotique)

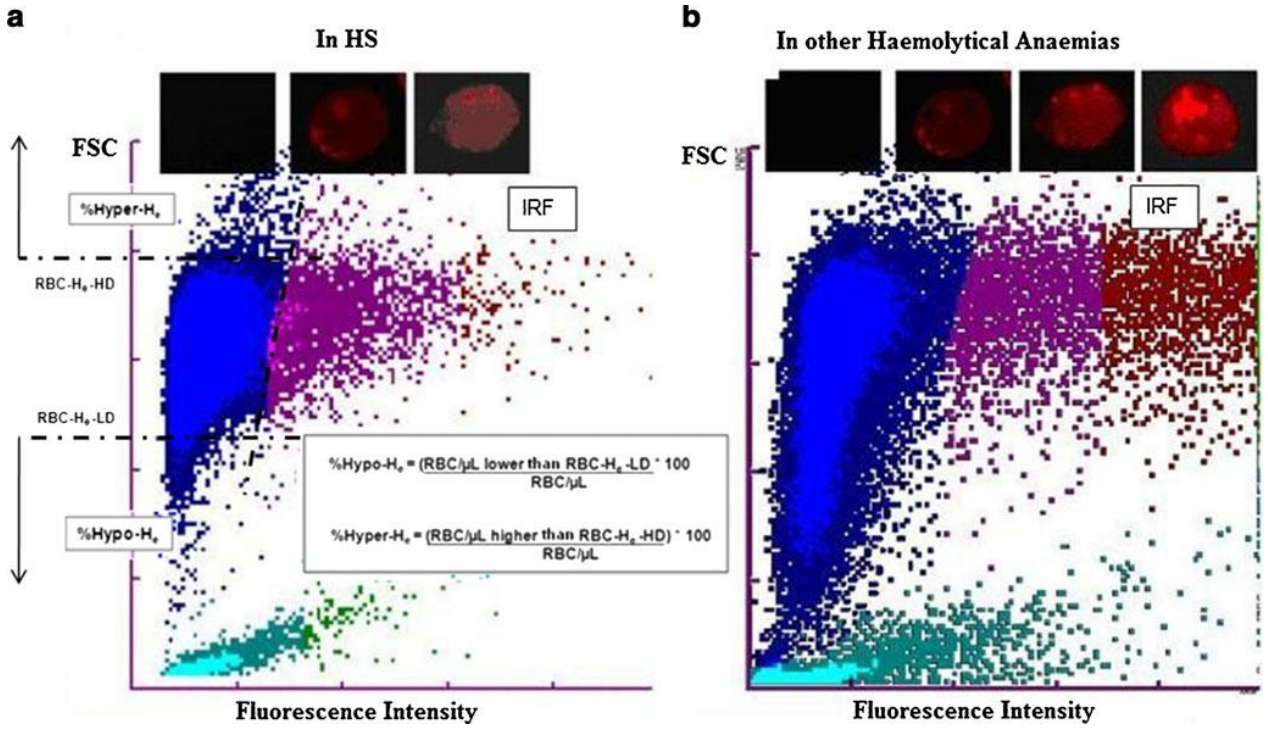
- SDS page
- ektacytométrie
- (génétique)

Morphologie



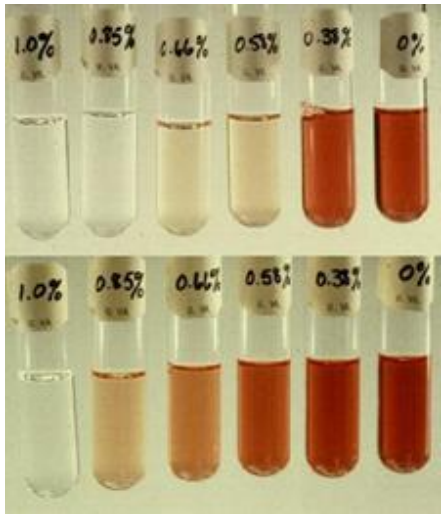
Indices

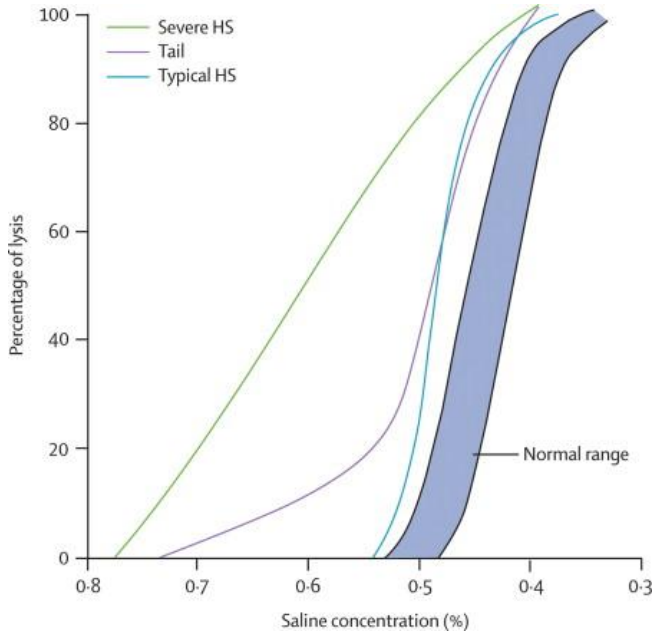
- MRV
- MSCV
- Ret/IRF



Ann Hematol 2011;90:759

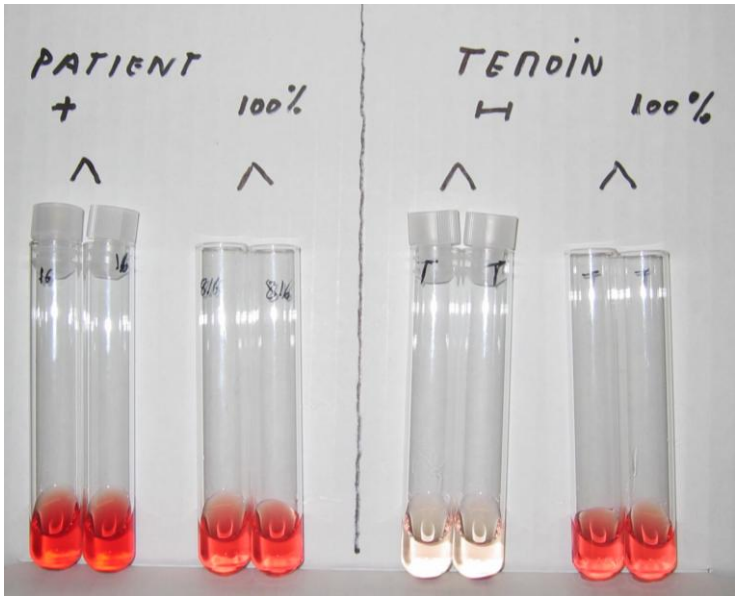
Résistance osmotique



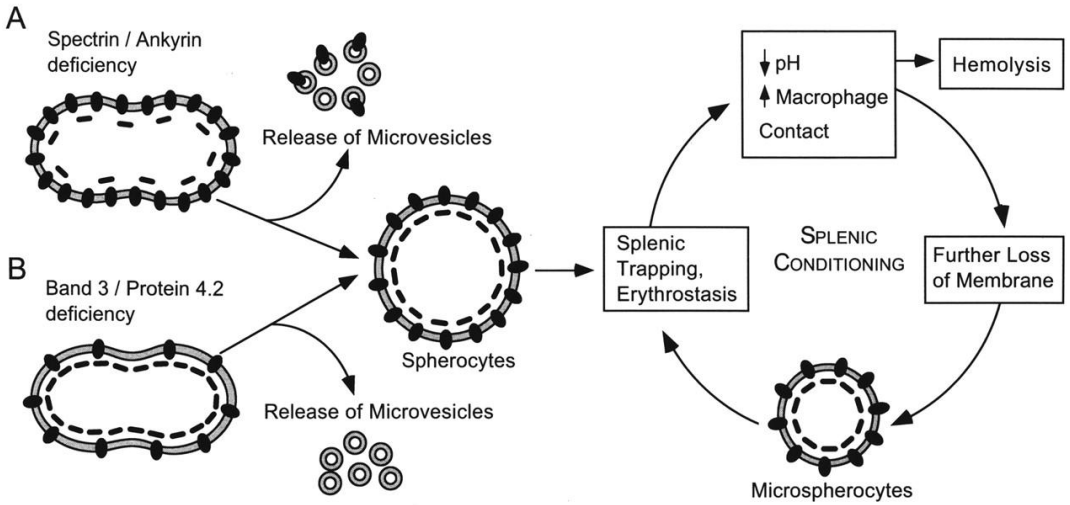


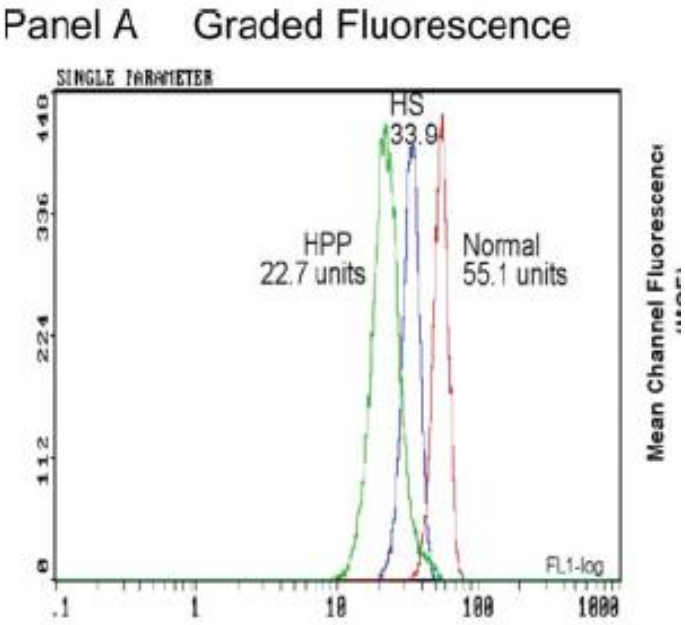
Lancet 2008;372:1411-26

Cryohémolyse hypertonique



Eosine-5-maléimide





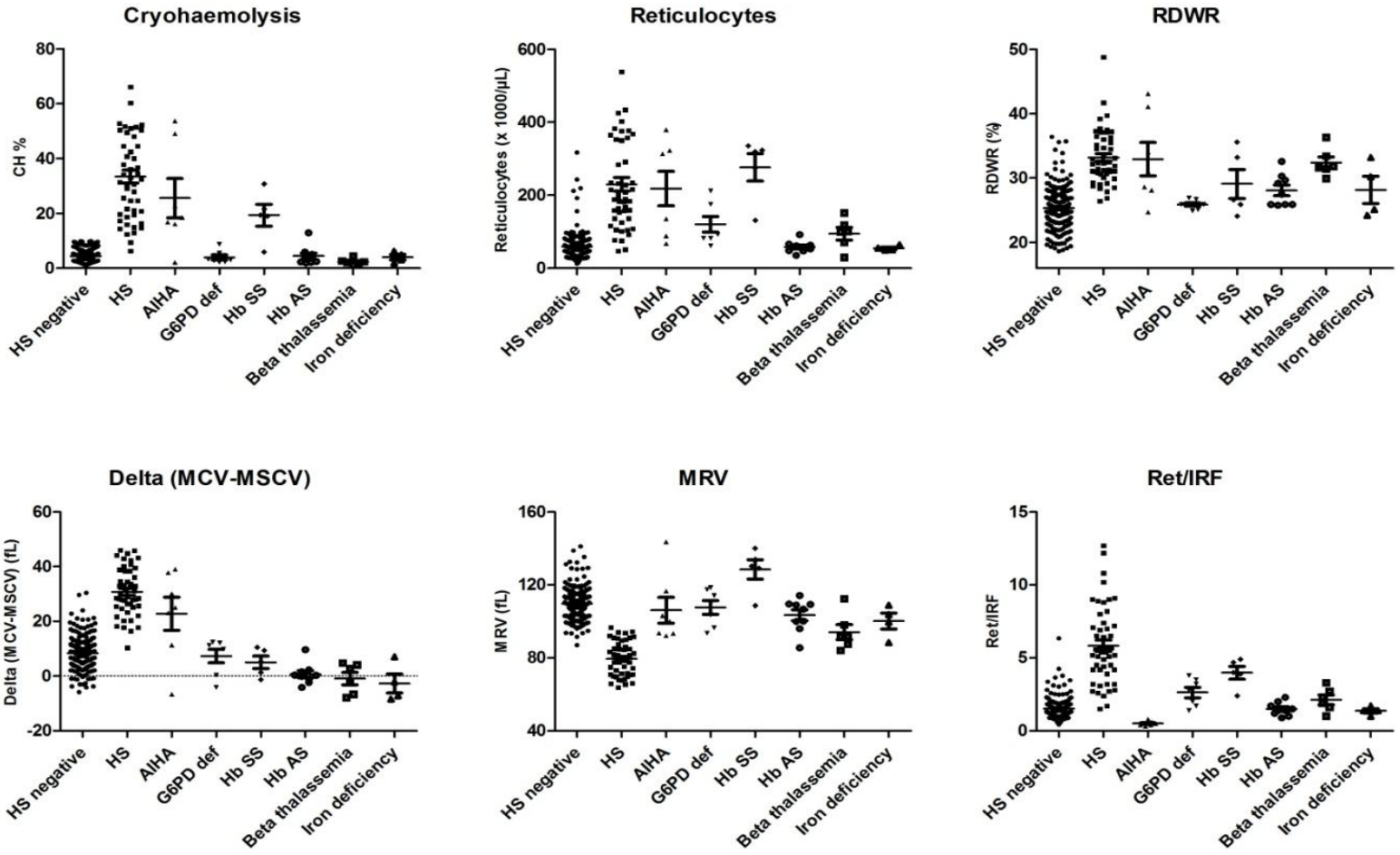
Cytometry B Clin Cytom 2008;74:244-50

Dépistage SH

Test	Sensitivity	Specificity	Reference(s)
Osmotic Fragility (OF)	Fresh = 68 % 24 h Incubn = 81 %	Not given Not given	Bianchi *et al*. 2012
Compensated HS:	Fresh = 53 % 24 h Incubn = 64 %	Not given	
Acid Glycerol Lysis Time (AGLT)	95%	Not given	Bianchi *et al*. 2012
Cryohemolysis	100 %	86 %	Streichman *et al* (1990)
EMA Binding	89 % - 99.1%	92.7 % - 99.1%	King *et al*. (2000) Stoya *et al* (2006) Girodon *et al*. (2007) Bianchi *et al* (2012)

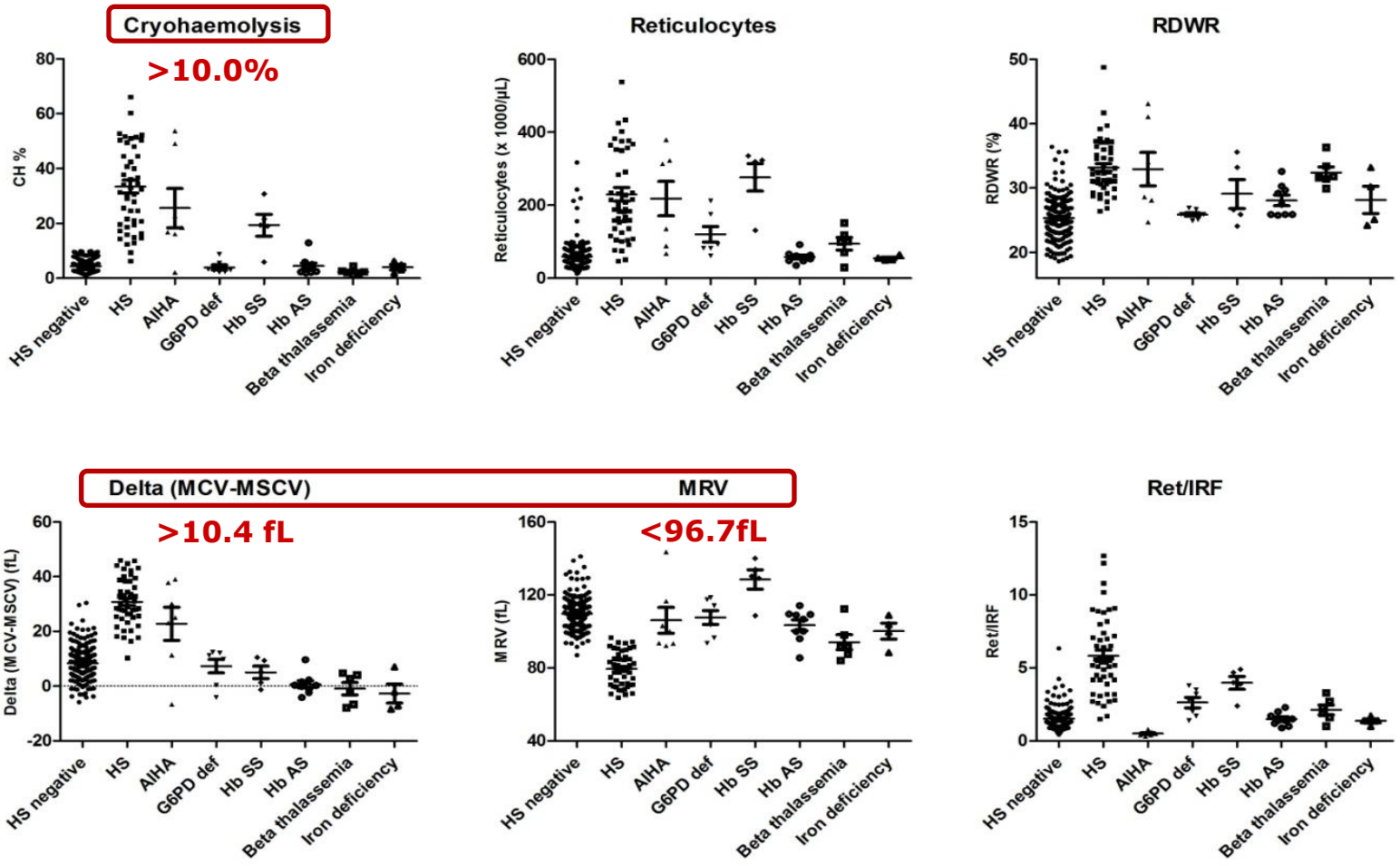
References: Bianchi *et al*. (2012) Haematologica **97**, 516
Streichman et al. (1990) Am J Hematol. **35**, 104. Stoya *et al*. (2006) Acta Haematol. **116**, 185.
King et al. (2000) Br J Haematol. **111**, 924. Girodon *et al*. (2007) Br J Haematol. **140**, 464.

Dépistage SH



Ann Hematol 2014;93:1809

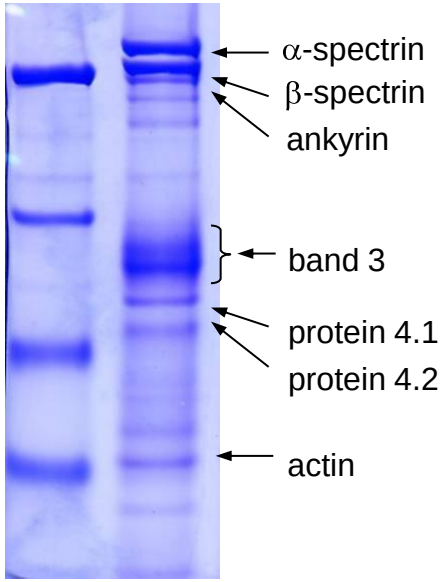
Dépistage SH



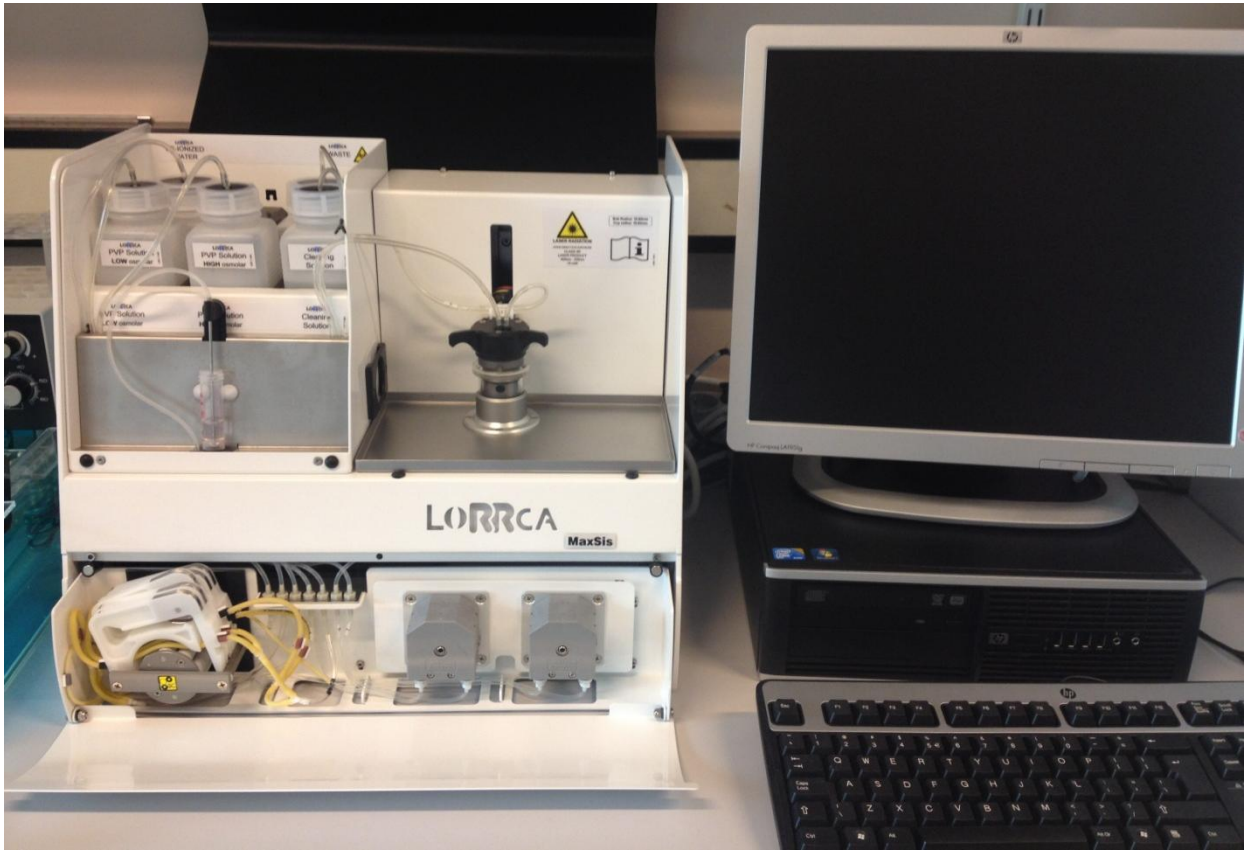
Ann Hematol 2014;93:1809

Diagnostic SH

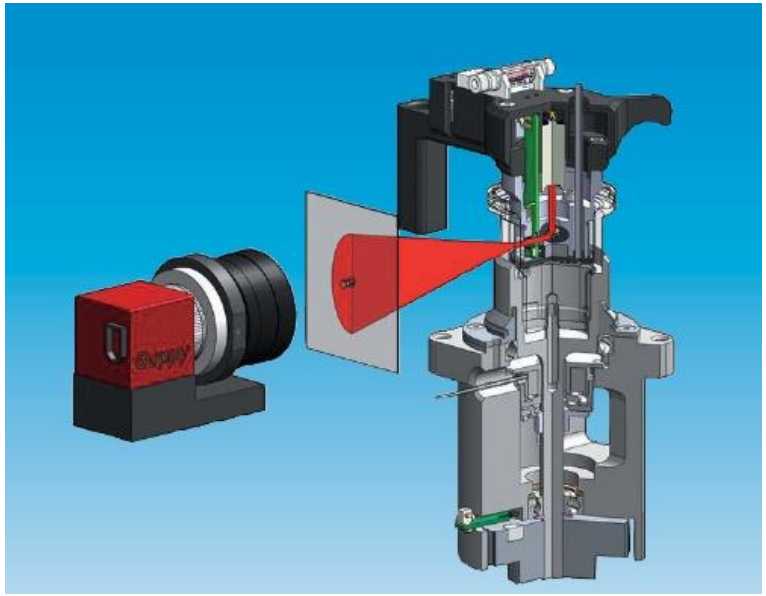
- sensibilité 60%
- mesure intensité déficience
- recommandée
  - phénotype plus sévère qu'attendu
  - résultats dépistage équivoques
  - avant splénectomie

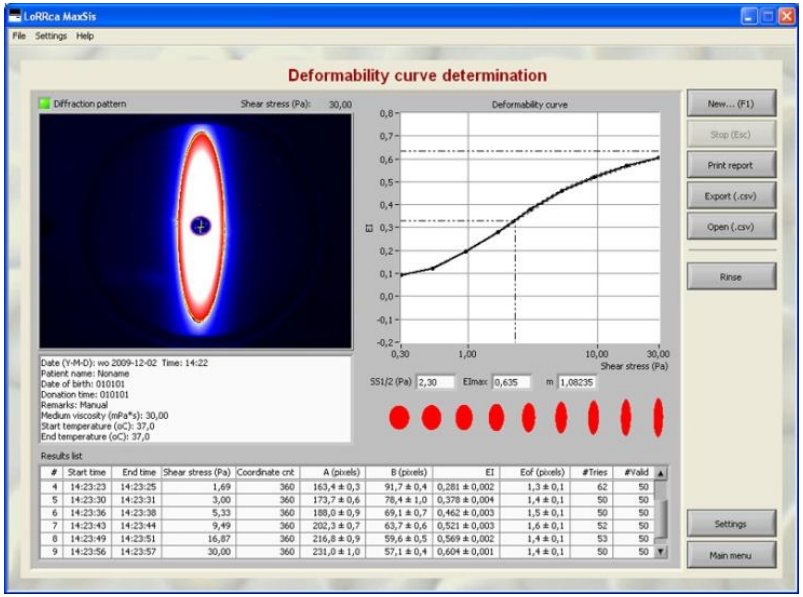


Diagnostic SH

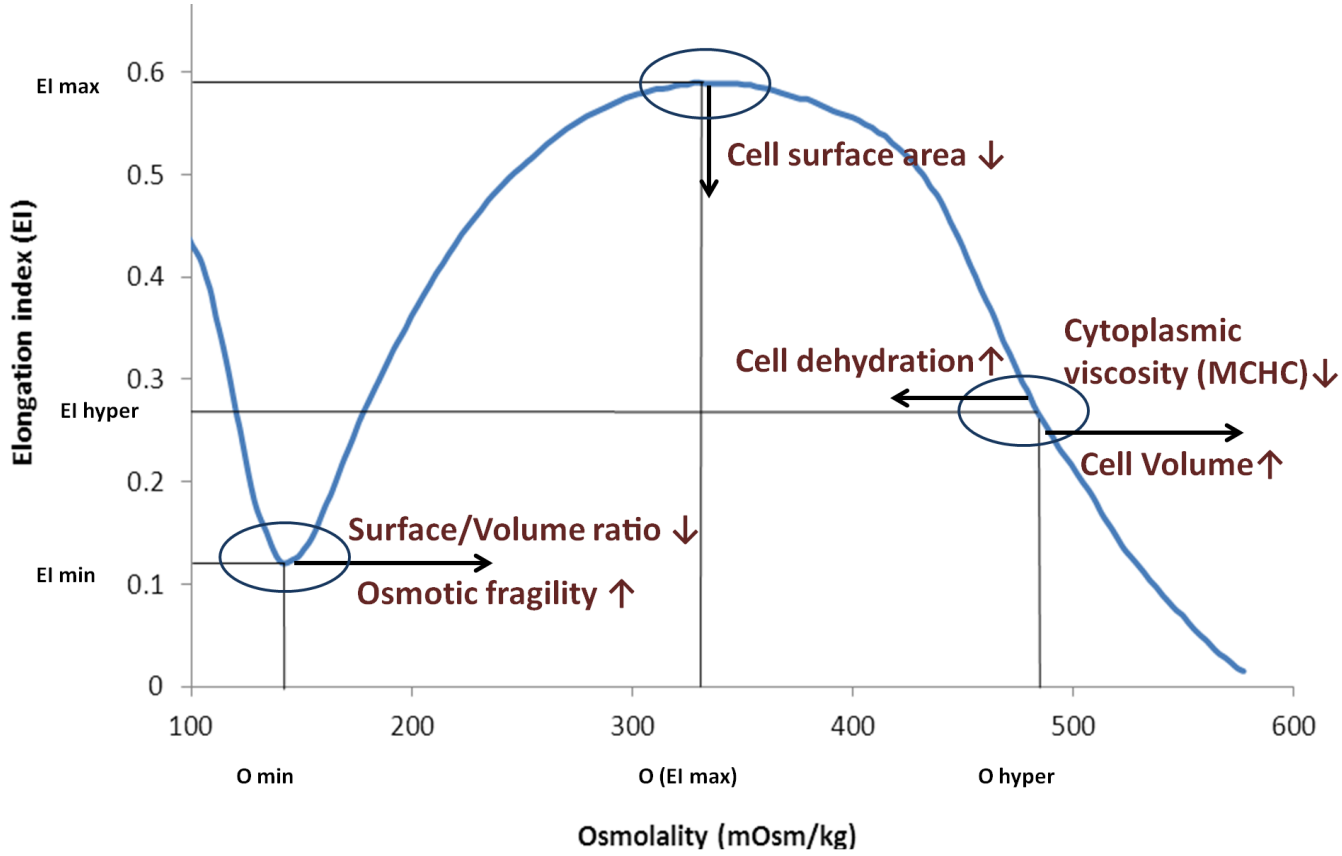


Ektacytométrie

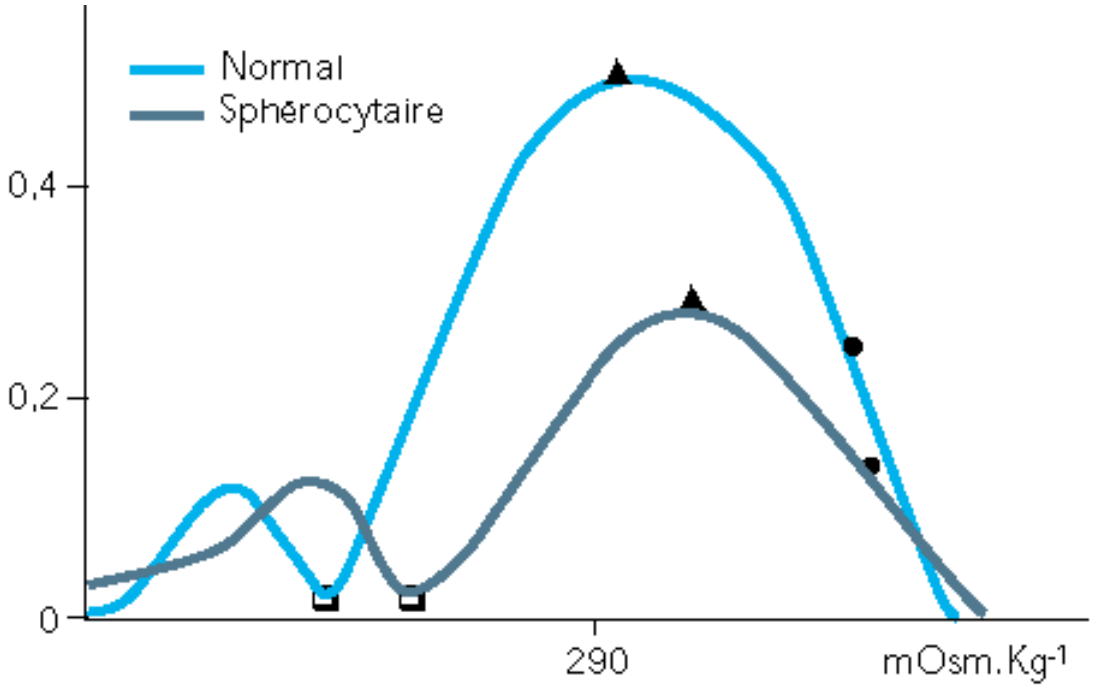




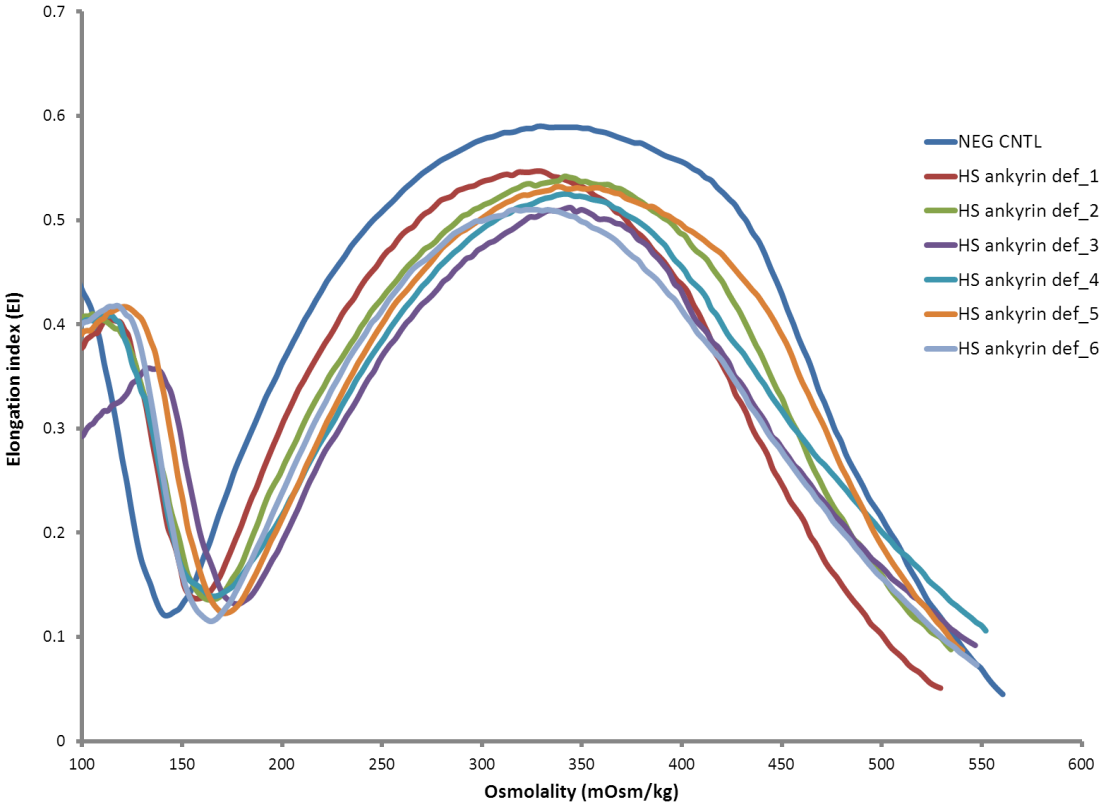
Ektacytométrie



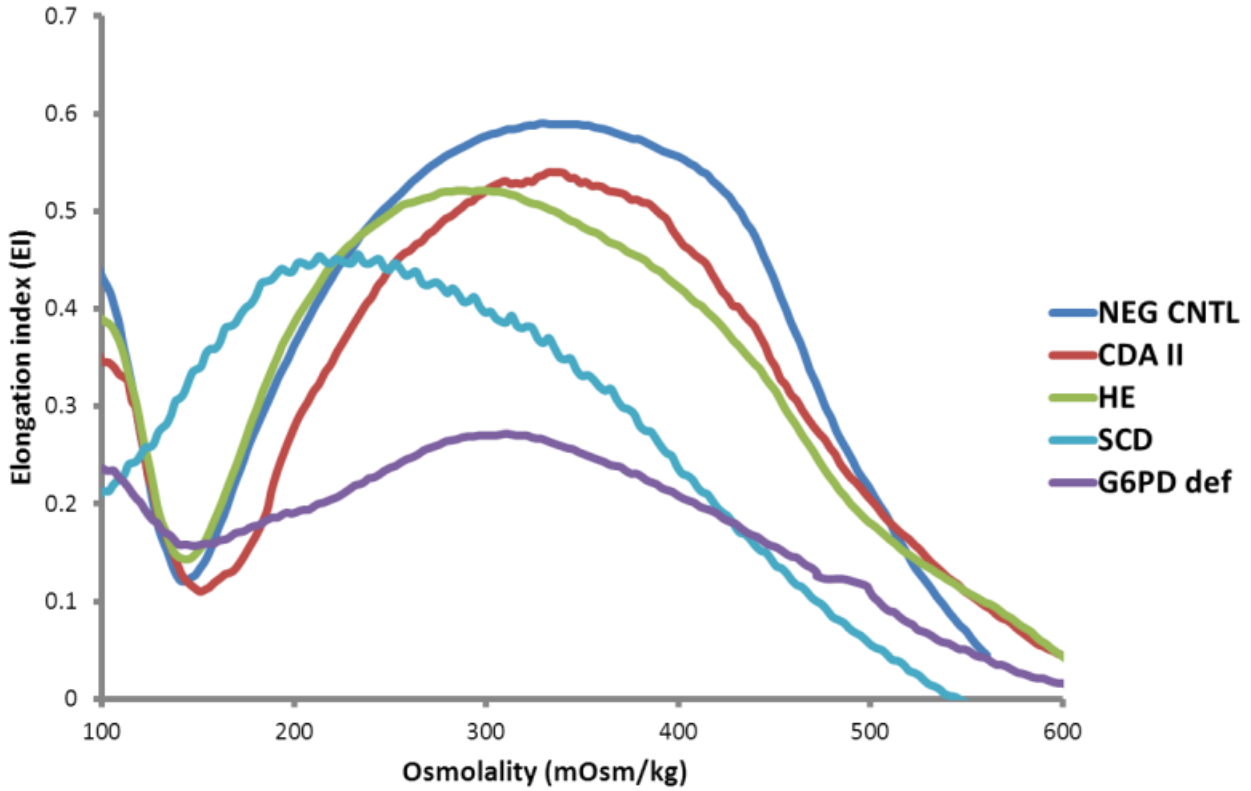
Ektacytométrie



Ektacytométrie



Ektacytométrie



Retourner maison

1. Hémolyse
2. Hémoglobinopathies
3. Enzymopathies érythrocytaires
4. Membranopathies
5. **Message de Noël**

Retourner maison

Examen hématologique			
Numération			
Leucocytes	▼3.2	4.2-11.4	x10^3/mm³
Globules rouges	▼3.03	3.8-5.2	x10^6/mm³
Hémoglobine	▼10.9	11.8-15.5	g/dL
Hématocrite	▼31.2	35.3-46.1	%
MCV	▲103.0	80.8-99.2	µm³
MCH	▲36.0	26.4-34.2	pg
MCHC	34.9	32-35.4	g/dL
RDW	▲14.1	10.9-13.4	%
Ind. de distr.des GR	49.4		
Réticulocytes	▲36.0	7.0-25.0	/1000 GR
Réticulocytes absolus	110.3	30.0-111.0	x10^3/mm³
Plaquettes	▼153	155-346	x10^3/mm³
MPV	7.5	6.4-10	µm³
PCT	0.115	0.115-0.4	%
PDW	16.7	15-18	
Volume reticulo. moyen	109.0		µm³
Fraction reticulo. immature	0.5		

Interprétation ?

Prendre un clinicien par la main

Merci !

LHUB-ULB

… et joyeuses fêtes ☺