ANALYTİCAL İNTERFERENCES - CONTİNUOUS CHALLENGE İN THE CLİNİCAL LABORATORY

Nora Nikolac

University Department of Chemistry, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia

March 28, 2015

expoMED 2015, Istanbul, Turkey

Key issues in interference management

Interference mechanisms

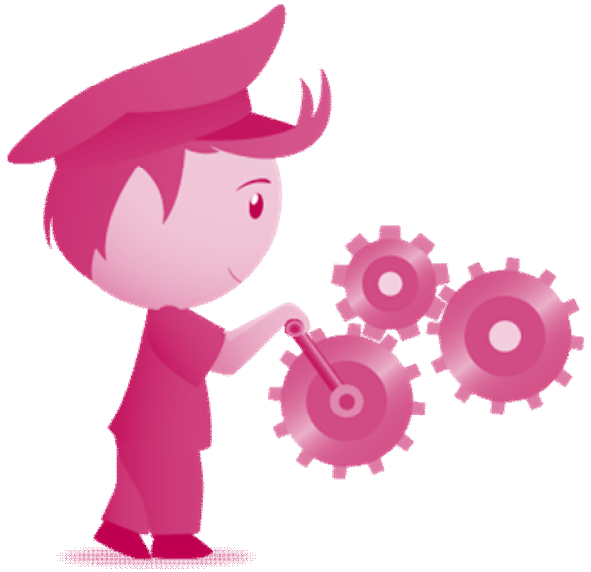
Detection of interferences

Measuring the effect of interferences

Dealing with samples

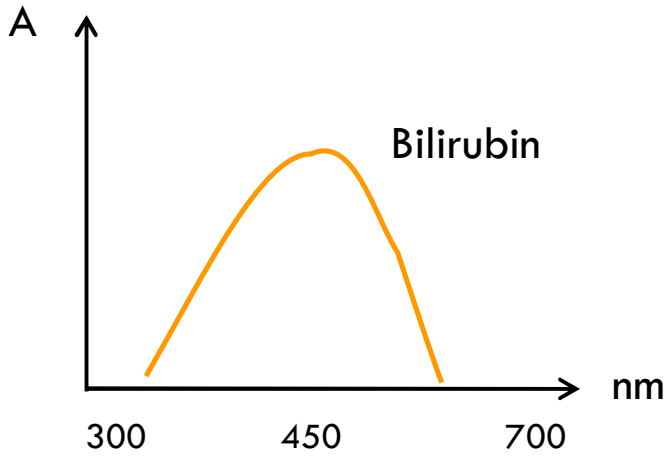
Is there any unknown territory?

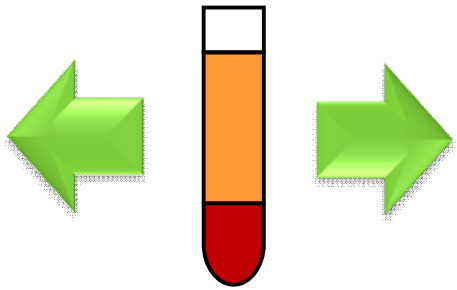
1 Interference mechanisms



Icteric sample

1. Spectrophotometric interference





Icteric sample

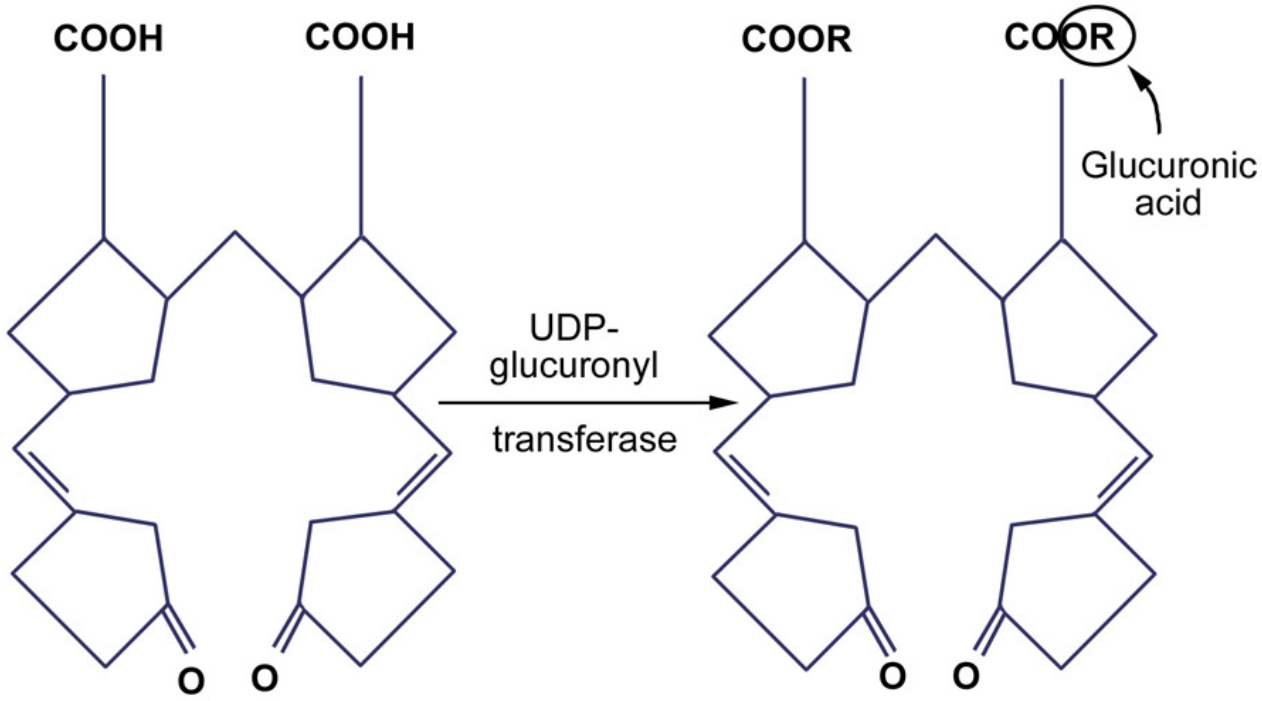
2. Chemical interference

Bilirubin + H_2O_2

H_2O_2/POX

Bilirubin $\xrightarrow{OH^-}$ Biliverdin

Differences in icteric sample



Unconjugated bilirubin

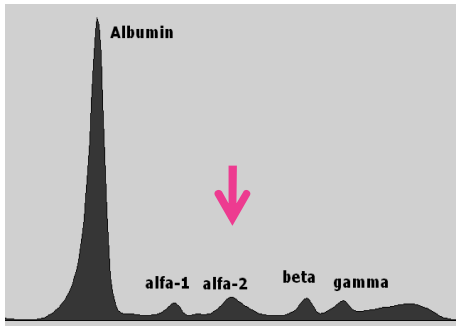
Oxidation in alkaline pH (creatinine)

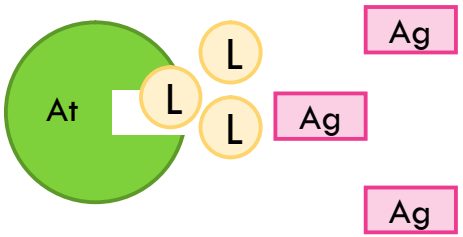
Conjugated bilirubin

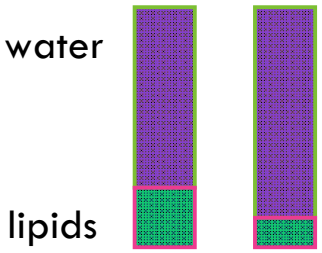
Inorganic phosphates

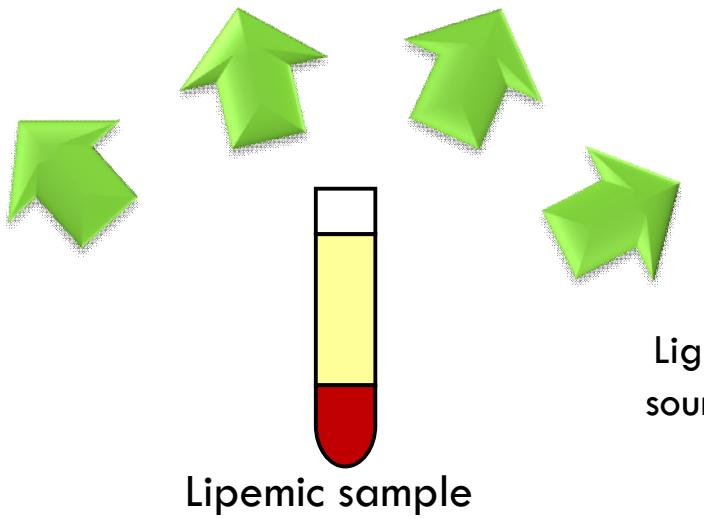
Lipemic sample

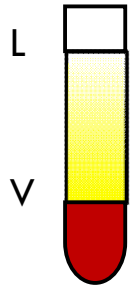
1. Physical and chemical interference
2. Volume displacement effect
3. Non-homogeneity
4. Spectrophotometric interference

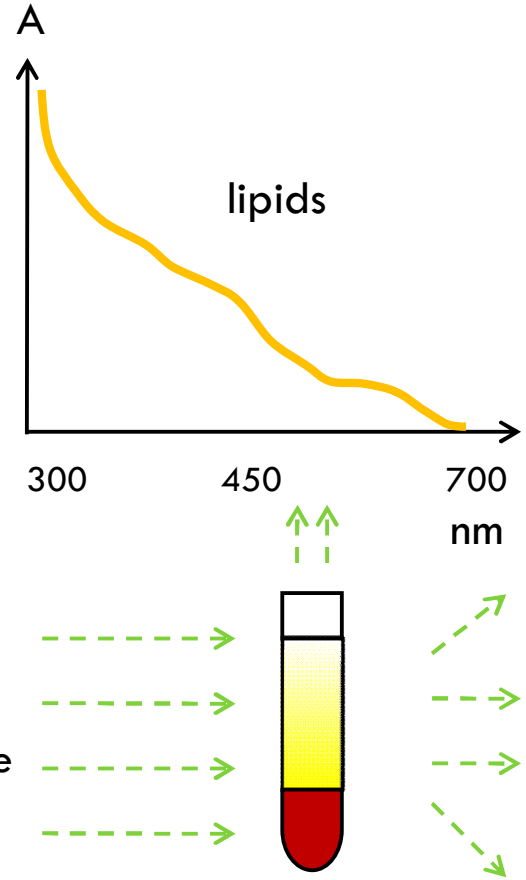








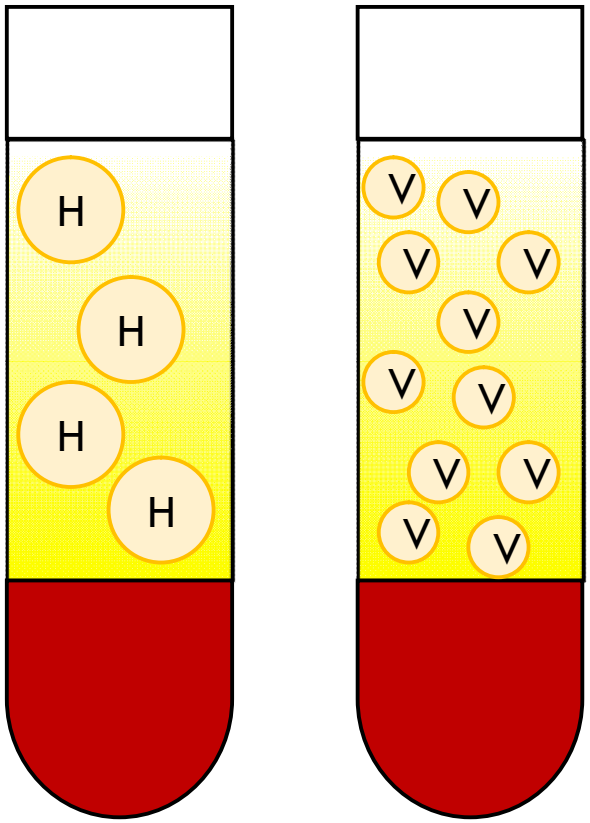




Differences in lipemic sample

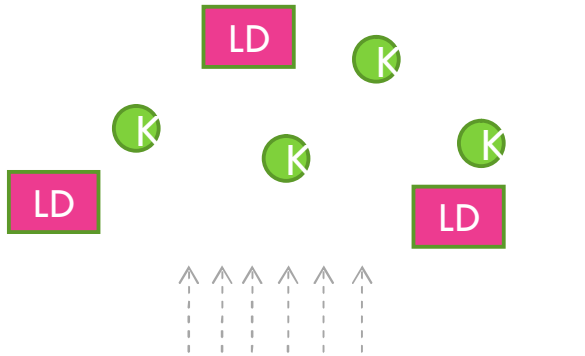
Same degree of turbidity! (L index)

Lipemia caused by larger chylomicrons



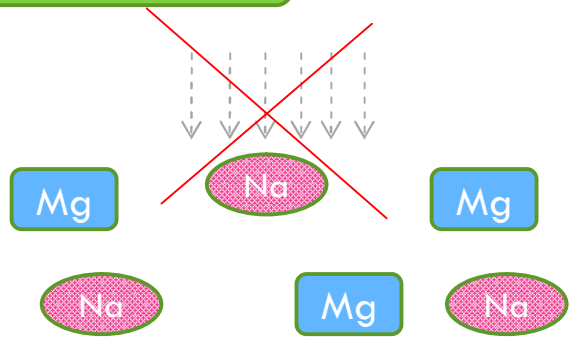
Lipemia caused by smaller VLDL

Hemolytic sample



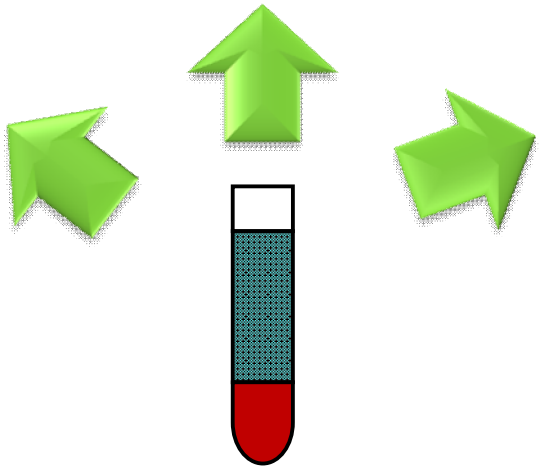
1. Accumulation

2. Dilution



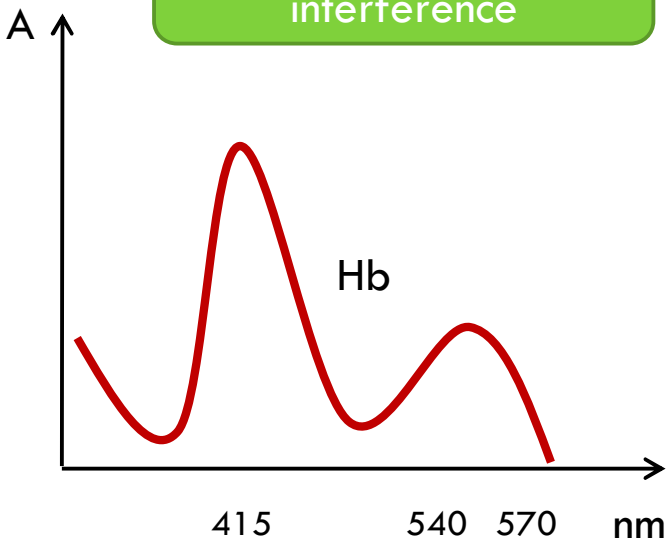
3. Chemical interference

Hemoglobin
Katepsin D
Adenylate kinase



Hemolytic sample

4. Spectrophotometric interference

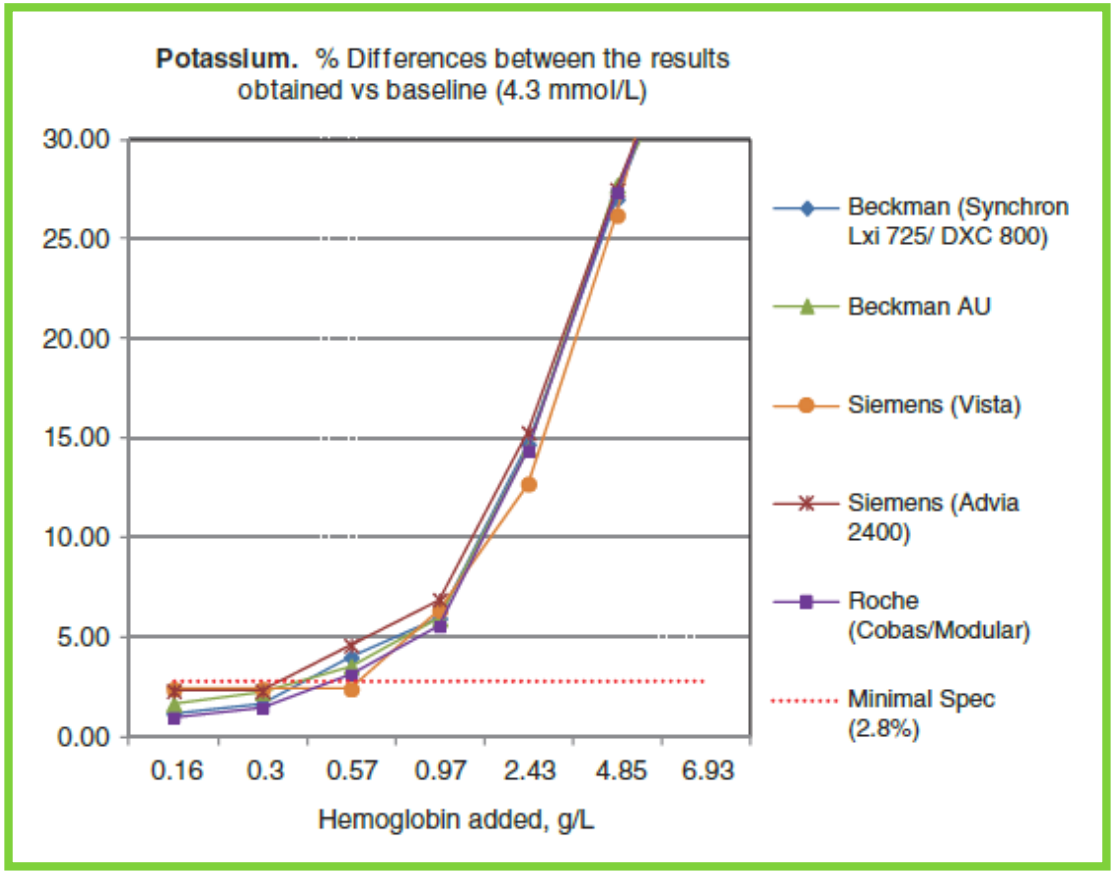


In vivo vs. in vitro hemolysis

In vitro	Parameter	*In vivo*
↑↑↑	LD	↑↑↑
↑↑↑	Free Hb	N/↑
N	Haptoglobin	↓
↑↑↑	K	N/↑
N	Reticulocytes	↑↑↑

Universal degree and direction

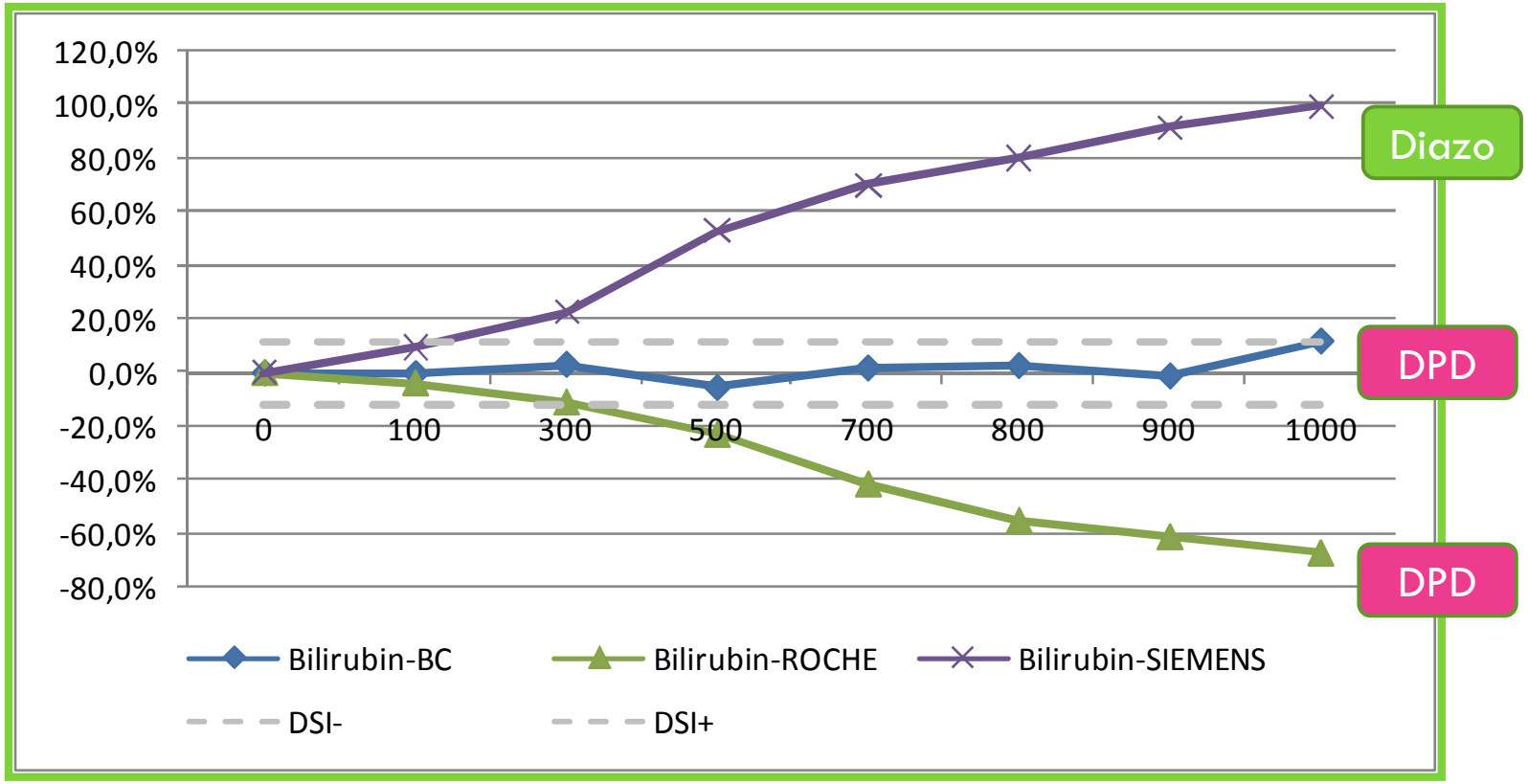
- **Potassium** and **hemolysis**



Fernandez P, et al. Harmonization in hemolysis detection and prevention. A working group of the Catalonian Health Institute (ICS) experience. Clin Chem Lab Med. 2014 Nov;52(11):1557-68.

Method and reagent specific interferences

- **Bilirubin** and **lipemia**



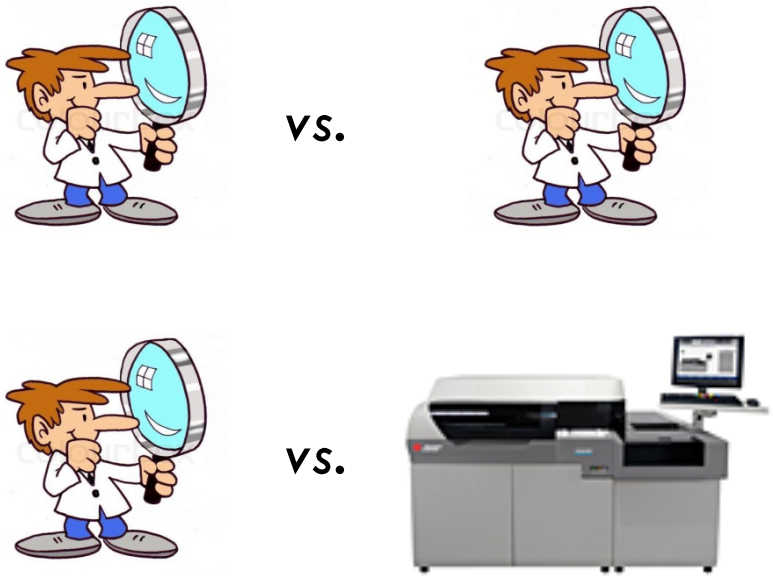
Nikolac N, Simundic AM, Miksa M, Lima-Oliveira G, Salvagno GL, Caruso B, Guidi GC. Heterogeneity of manufacturers' declarations for lipemia interference – urgent call for standardization. Clin Chim Acta 2013;426:33-40.

2 How to detect interferences

Visual detection

Clin Chem Lab Med 2009;47(11):1361–1365 © 2009 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2009.306

Comparison of visual vs. automated detection of lipemic, icteric and hemolyzed specimens: can we rely on a human eye?



	Kappa (95% CI)
Lipemia	0.70 (0.63–0.77)
Hemolysis	0.62 (0.54–0.70)
Icteria	0.48 (0.24–0.72)

	Kappa (95% CI)
Lipemia	0.56 (0.42–0.69)
Hemolysis	0.53 (0.43–0.63)
Icteria	0.64 (0.57–0.71)

Simundic AM, Nikolac N, et al. Comparison of visual versus automated detection of lipemic, icteric and hemolyzed specimens: can we rely on a human eye? Clin Chem Lab Med 2009;47(11):1361-5.

Serum indices

- Standardization
- Negligible cost
- Negligible TAT increase

Clinical Biochemistry 46 (2013) 1281–1284

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Systematical assessment of serum indices does not impair efficiency of clinical chemistry testing: A multicenter study

Giuseppe Lippi [a,*], Paola Avanzini [a], Daniele Campioli [b], Giorgio Da Rin [c], Mariella Dipalo [a], Rosalia Aloe [a], Davide Giavarina [d], Gian Luca Salvagno [e]

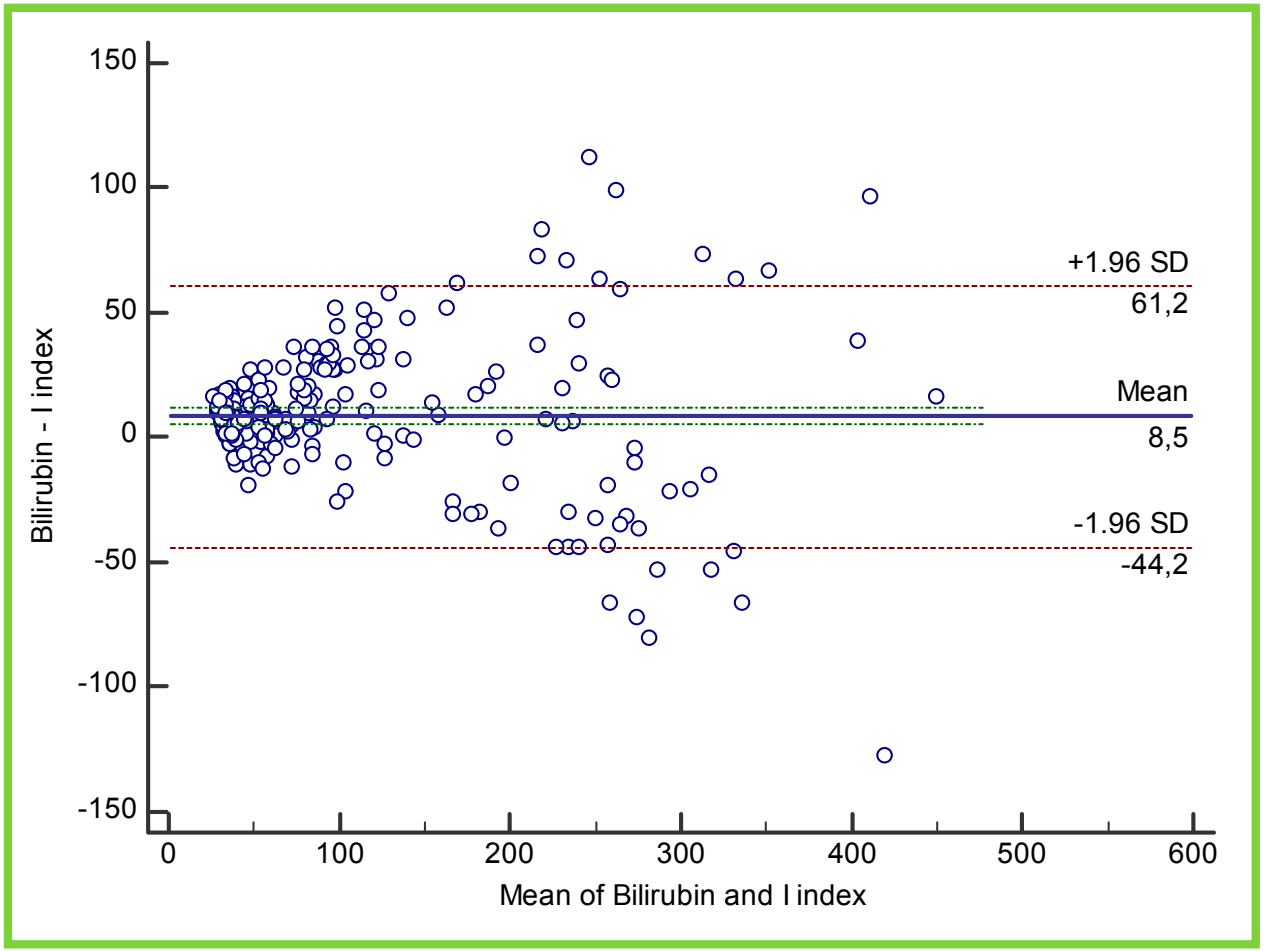
TAT on 5 analytical platforms: −0.2 to +5.0% (−3 to +85 s)

Verification of the serum indices

- Precision
- Accuracy
  - L index: Amount of added Intralipid® (concentration of TG)
  - I index: Amount of added bilirubin (concentration of bilirubin)
  - H index: Concentration of hemoglobin
- Comparability
- Cross-reactivity
- IQM – no calibrators and controls!

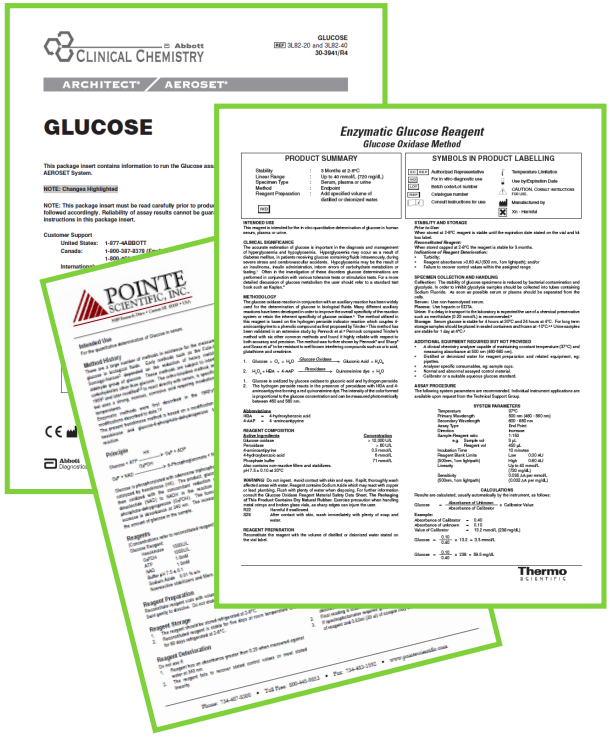
Accuracy

- I index and bilirubin, Abbott Architect c8000

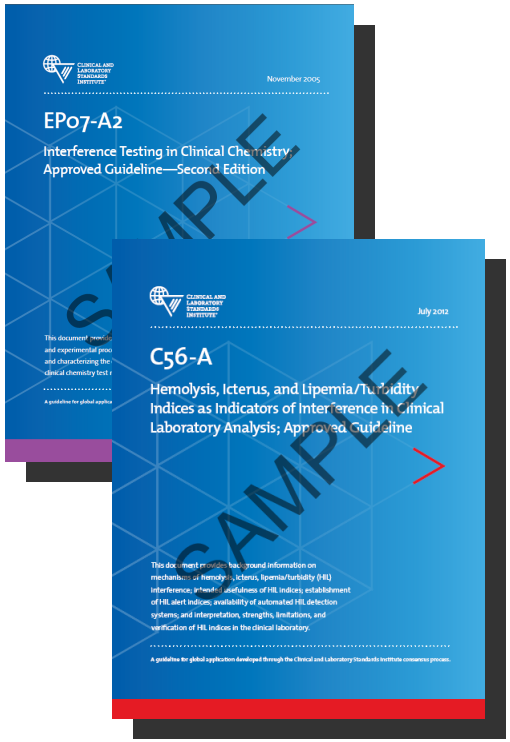


3 How to measure the effect of interferences

Investigation protocols



Manufacturers claims



CLSI documents

Verification of data

1. Acceptance criteria

1. Goals based on analytical performance related to clinical outcomes

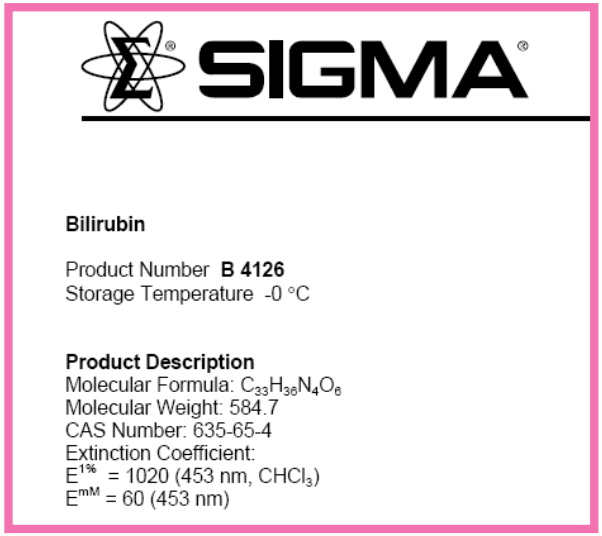
2. Goals based on biological variation

	Criteria based on biological and analytical variability				Manufacturers		
	2 x SD	CVw	DSI	TE	BC	R	S
Potassium	1.6%	4.8%	2.4%	2.2%	Not declared	10%	10%
Bilirubin	2.1%	23.8%	11.9%	19.1%	10%	10%	20%

Manufacturers use arbitrary criteria of 5% or 10%!

2. Choosing the interferent - Icteria

- **Adding bilirubin powder into clear samples (CLSI)**
- Conjugated and unconjugated bilirubin differ in effects
- Difficult dissolving of bilirubin
- Alkaline solution can change pH of the sample



SIGMA

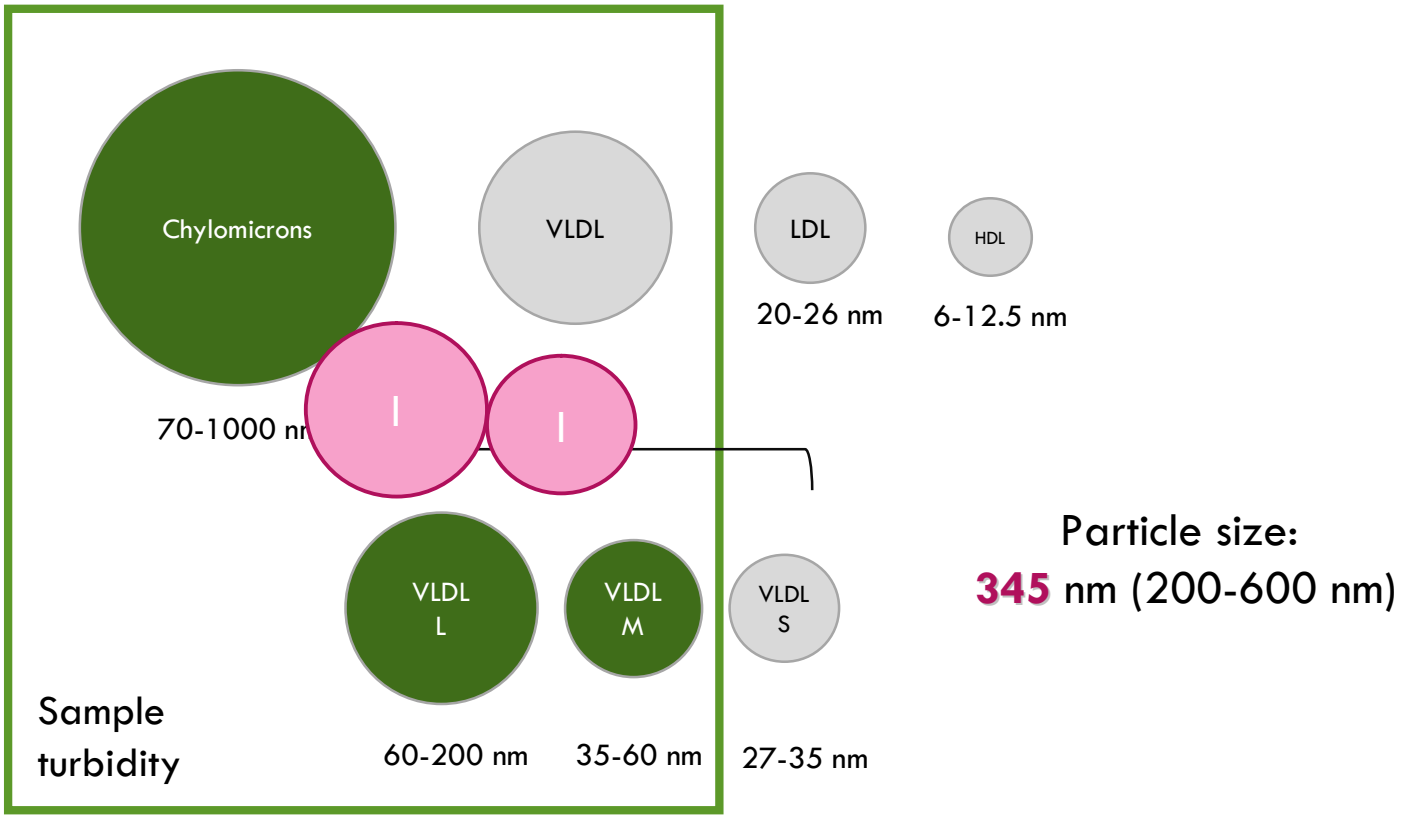
Bilirubin

Product Number **B 4126**
Storage Temperature -0 °C

Product Description
Molecular Formula: $C_{33}H_{36}N_4O_6$
Molecular Weight: 584.7
CAS Number: 635-65-4
Extinction Coefficient:
$E^{1\%}$ = 1020 (453 nm, $CHCl_3$)
E^{mM} = 60 (453 nm)

2. Choosing the interferent - Lipemia

- **Adding Intralipid® solution into clear samples (CLSI)**
- Synthetic fat emulsion used for parenteral diet



Intralipid induced lipemia differs from native lipemia!

2. Choosing the interferent - Lipemia

- **Spiking clear samples with the lipemic patient pool**

- Large amount of pool
- Freezing changes properties of particles
- Heterogeneous lipemic pool (different lipoproteins)
- Failure to replicate study

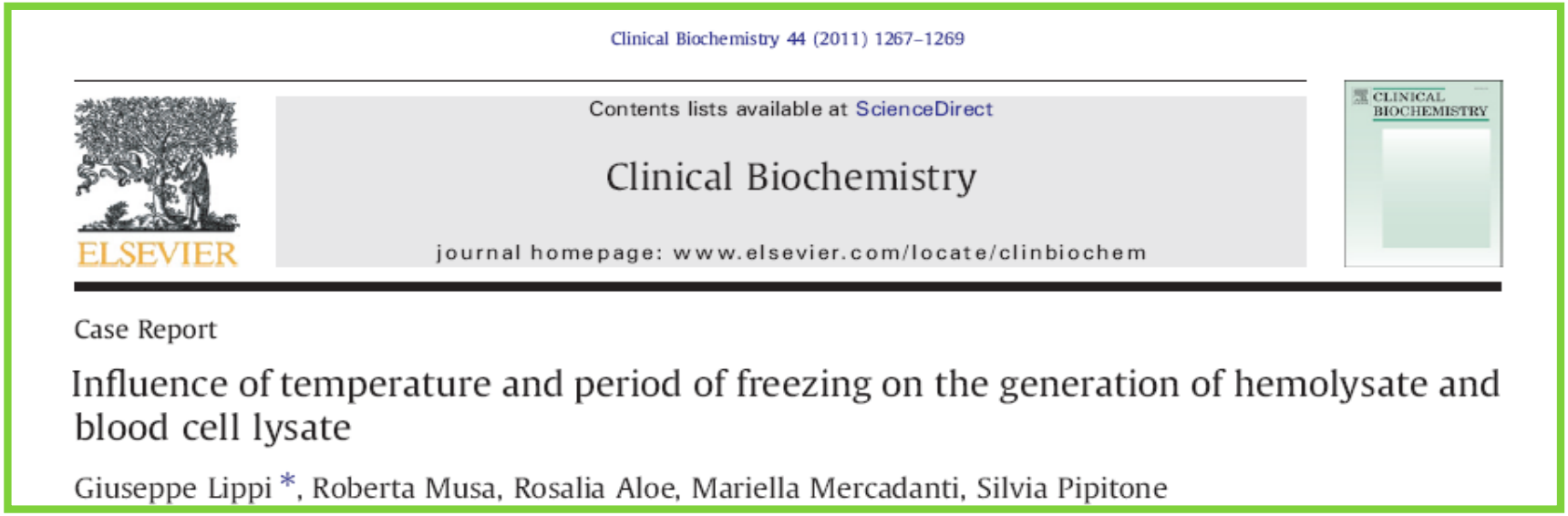
2. Choosing the interferent - Hemolysis

- **Adding hemolysate prepared by osmotic shock procedure into clear sample (CLSI)**

- All mechanisms of interference can't be tested by this protocols
  - analytes accumulating in the cell in pathological samples
  - some drugs

2. Choosing the interferent - Hemolysis

- **Freezing of the sample**



Clinical Biochemistry 44 (2011) 1267–1269

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Case Report

Influence of temperature and period of freezing on the generation of hemolysate and blood cell lysate

Giuseppe Lippi *, Roberta Musa, Rosalia Aloe, Mariella Mercadanti, Silvia Pipitone

12 hours at -20°C or 2 hours on -80°C

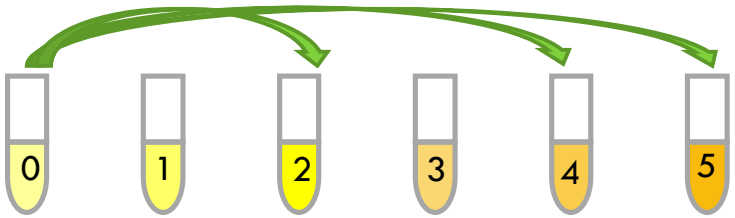
Can't be used for analytes that can't be freezed!

2. Choosing the interferent - Hemolysis

- **Aspiration of full blood using syringe**

- Syringe diameter and number of replicates can create good hemolysis scale
- Imitates hemolysis in vitro
- Lyses other cells (i.e. leukocytes)

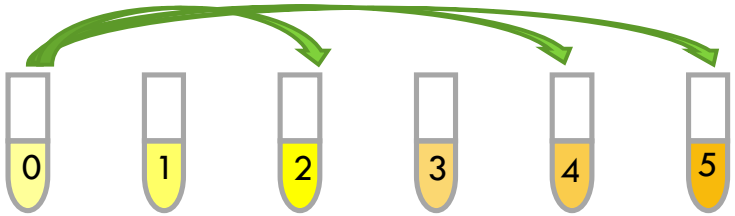
3. Choosing the analyte concentrations



At least 6 interferent concentrations (0 + 5)

Low or normal analyte concentration

CLSI EP7-A2 Appendix B



High or critical analyte concentration

	Interferent concentrations					
	0	C1	C2	C3	C4	C5
Hemoglobin (g/L)	0	0.5	1.25	2.5	5.0	10.0
Intralipid (mg/dL)	0	100	300	500	1000	2000
Bilirubin (µmol/L)	0	43	120	257	513	1026

4. Reporting the results

- Manufacturers should declare:
  - Materials used to create interferences
  - Interferent concentration
  - Analyte concentration
  - Allowable error

Unstandardized and incomplete data!

Reagent, manufacturer	Hemolysis information
ACE (Bulhman Laboratories)	Do not analyze
Acid phosphatase (Sentinel)	No interference up to 0.15 g/dL Hb
Ammonia (Randox)	NH_3 = 50 µmol/L: No interference up to 125 mg/dL Hb NH_3 = 300 µmol/L: No interference up to 250 mg/dL Hb
Glucose (Abbott)	Glucose = 4.3 mmol/L: 4.4% (10 g/L Hb), 8.3% (20 g/L Hb) Glucose = 6.6 mmol/L: 1.7% (10 g/L), 4.0% (20 g/L)
Lactate (Beckman Coulter)	< 5% up to 5 g/L Hb
Cholinesterase (Ortho Clinical Diagnostics)	< 0.3 U/mL up to 150 mg/dL Hb

5. Verification of results

Clinica Chimica Acta 426 (2013) 33–40

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Heterogeneity of manufacturers' declarations for lipemia interference — An urgent call for standardization

Nora Nikolac [a,*,1], Ana-Maria Simundic [a], Manuela Miksa [a], Gabriel Lima-Oliveira [b,1], Gian Luca Salvagno [b], Beatrice Caruso [c], Gian Cesare Guidi [b,c]

[a] University Department of Chemistry, Medical School University Hospital Sestre Milosrdnice, Vinogradska 29, 10000 Zagreb, Croatia
[b] Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona, 37126 Verona, Italy
[c] Laboratory of Clinical Biochemistry and Hematology, Borgo Trento Hospital, P. le Aristide Stefani 1, 37126 Verona, Italy

Failure to verify manufacturers claims for lipemia and hemolysis!

DE GRUYTER

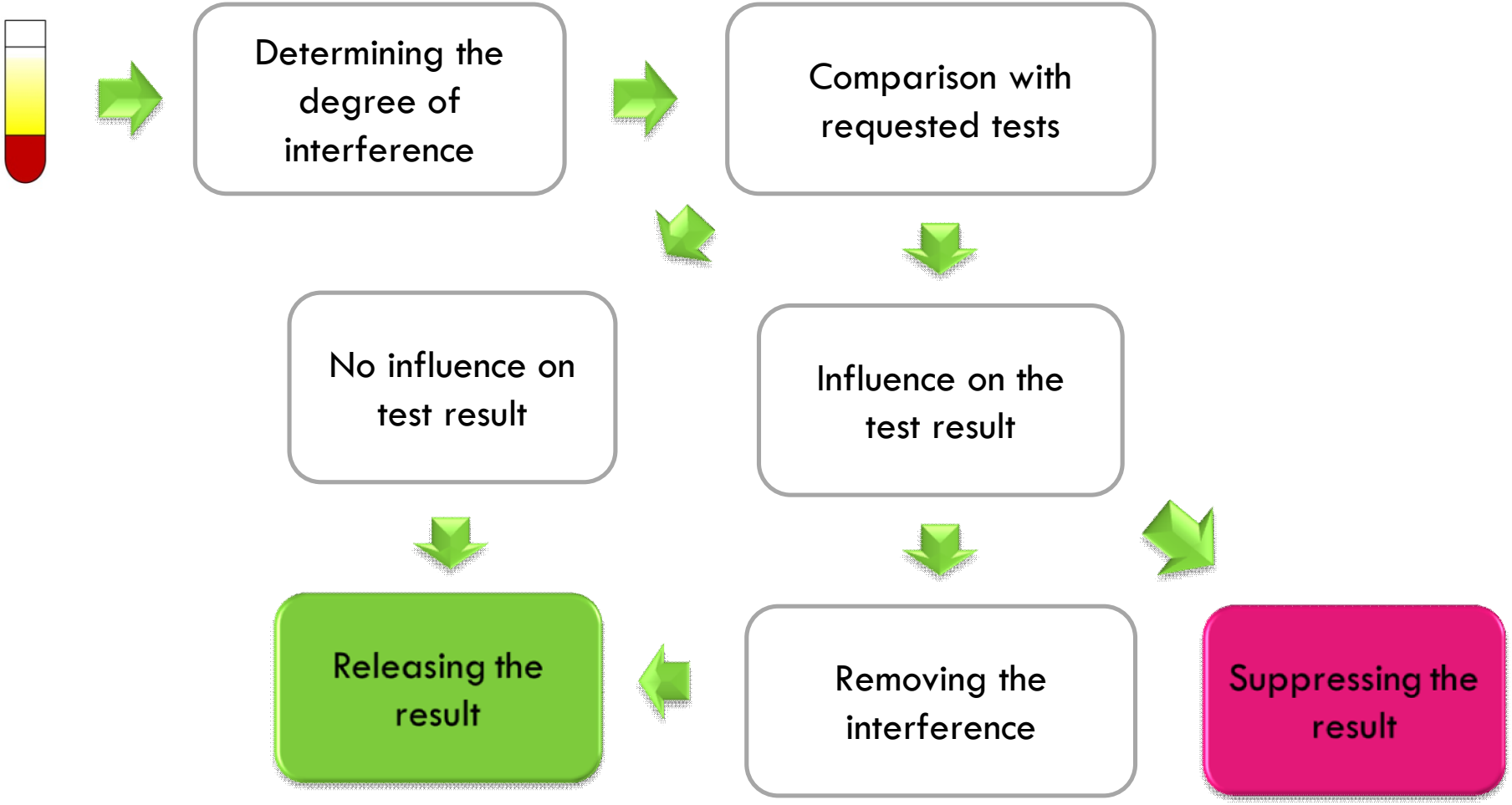
Clin Chem Lab Med 2014; 52(11): 1557–1568

Pilar Fernandez, María Antonia Llopis, Carmen Perich*, Maria Jesús Alsina, Virtudes Alvarez, Carmen Biosca, Gloria Busquets, Maria Vicenta Domenech, Rubén Gómez, Isabel Llovet, Joana Minchinela, Rosa Pastor, Rosa Ruiz, Ester Tarrés, Mercè Ibarz, Margarita Simón and Mercè Montesinos

Harmonization in hemolysis detection and prevention. A working group of the Catalonian Health Institute (ICS) experience

4 How to deal with samples

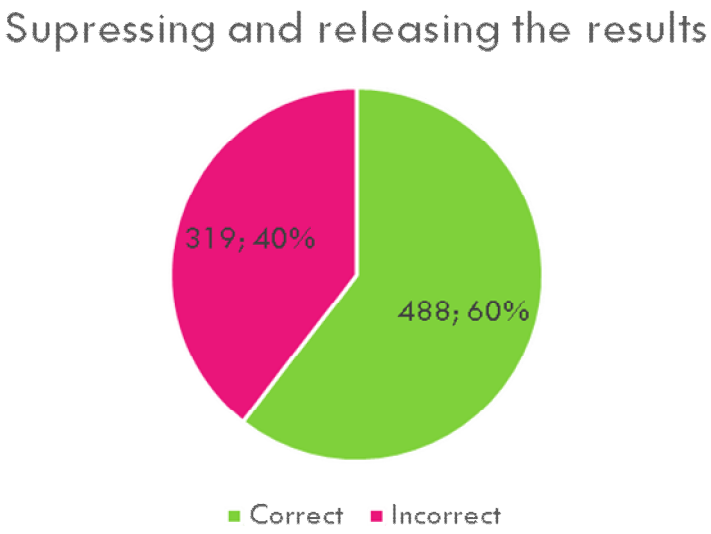
Dealing with unsuitable samples



Manual vs. Automatic protocol

Retroactive analysis of the manual protocol:

- 1 week , 4443 samples, 807 hemolysed samples (18.1%)



Implementation of automatic protocol

- Suppressing all tests influenced by hemolysis
- Releasing all tests not influenced by hemolysis

Unpublished data

Key issues in interference management

Interference mechanisms

Carefully consider potential mechanisms present in the sample

How to detect interferences

Know limitations of the method used for detection

How to measure the effect of interferences

Modify protocols for investigation

Question anything declared in manufacturers claims

How to deal with samples

Implement optimal protocols to assure best patient care

The dark side of the moon

Thank you on your attention.