Protein C is disproportionately affected by hyperbilirubinemia compared to other chromogenic assays on the Sysmex CS-2500

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Disclosures

- No disclosures

Hyperbilirubinemia in the coagulation laboratory

Chen 2015	Spiked normal plasma	CS-5100 CS-7000	PT aPTT D-Dimer	No significant differences
Nougier 2019	Spiked normal plasma	ACLTOP STAR	PT aPTT Fibrinogen	< 30mg/dl bilirubin- No significant bias ≥30mg/dl significant negative and positive bias
Mountari 2020	Spiked normal plasma	Cobas t511	PT aPTT Fibrinogen Anti-thrombin D-dimer	>30mg/dl significant differense on all tests assays
Scalambrino 2023	CLD patients with varying bilirubinlevels	ACLTOP 500 Thrombotrack	PT aPTT	No significant differences between chromogen and clotassay

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ORIGINAL ARTICLE

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Interference in specialized coagulation assays affecting the protein C pathway: Effects of marked haemolysis, hyperbilirubinaemia and lipaemia on chromogenic and clotting tests on two coagulation platforms

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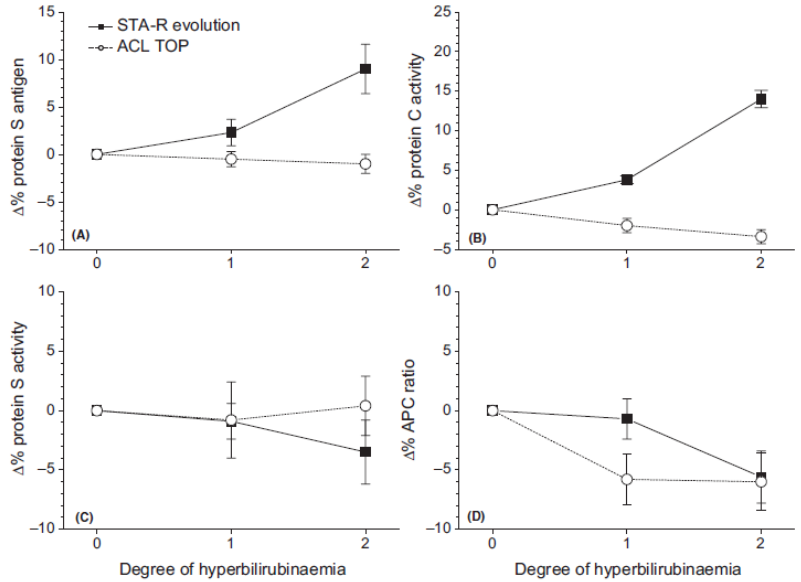


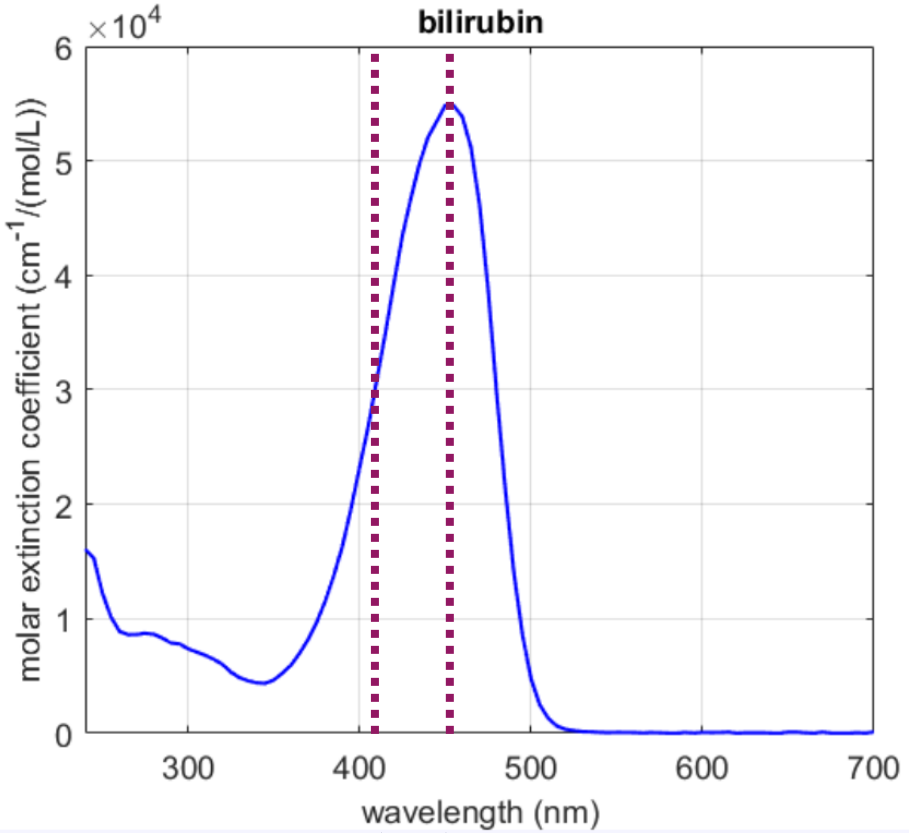
0 = <1.3mg/dl
1 = 8-10mg/dl
2 = 15-30mg/dl

≥ 8-10mg/dl (137-171µmol/L) Protein S Ag and Protein C significant bias
≥15-30mg/dl (256-513µmol/L) Protein S activity significant bias

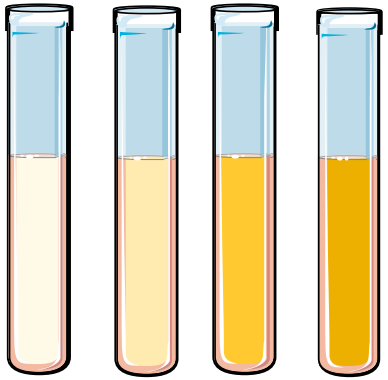
The assays evaluated on Sysmex CS-2500

Target	Assay	Wavelength	Bilirubin interference by manufacturer
Protein S, free	INNOVANCE® Free PS Ag, Siemens	800nm	Up to 1360 µmol/l
Protein C	Berichrom® Protein C, Siemens	405nm	Not reported
Factor VIII	Coamatic Factor VIII, Chromogenix	405nm	Not reported
Factor IX	BIOPHEN Factor IX, Hyphen BioMed	405nm	Up to 1026 µmol/l
Antithrombin	INNOVANCE® Antithrombin, Siemens	405nm	Up to 1026 µmol/l

Protein S to act as an ”anchorassay” that should be unaffected by bilirubin due to detection at a high wavelenght.



Artificial bilirubin interference



Pooled normal plasma
Bilirubin ditaurate (10000 µmol/l)
50 µmol/l 600 µmol/l
Concentration verified on Cobas Pro

Coagulation assay

Duplicats for all sampels
Fresh and frozen spiked samples
Free Protein S, Protein C, Factor VIII, Factor IX and Antithrombin

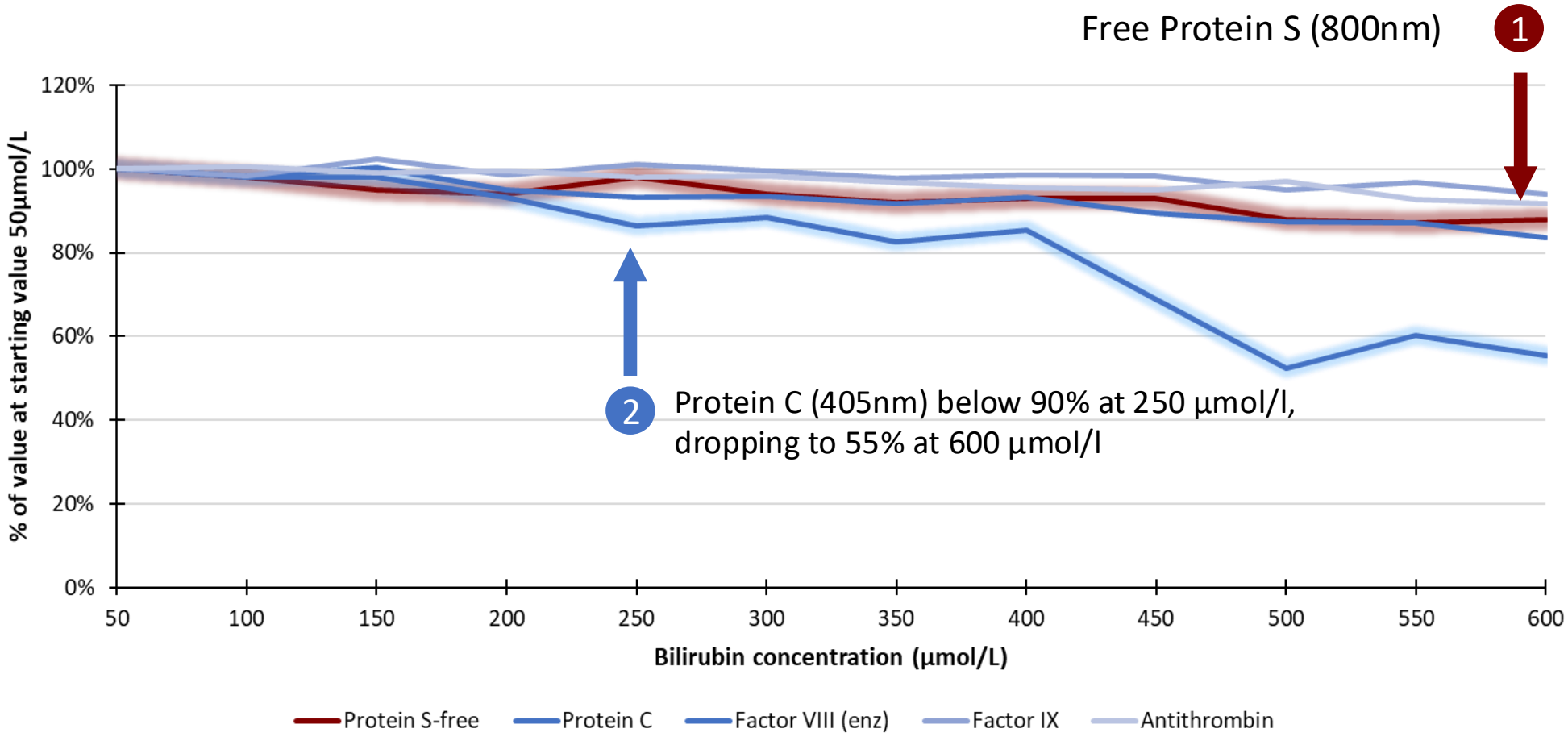
Clinical bilirubin data

lab_id	ror_id	Analy	resulta	bestallande_kombika
1122659433	11226594330013	BIL	4	11002203V02
1122659433	11226594330082	PS-fri	0,53	11002203V02
1123811963	11238119630013	BIL	7	11010151M01
1123811963	11238119630082	PS-fri	1,06	11010151M01
1124108219	11241082190013	BIL	8	11002151V02
1124108219	11241082190082	PS-fri	0,94	11002151V02
1124281966	11242819660013	BIL	4	11002151V02
1124281966	11242819660082	PS-fri	0,77	11002151V02

LIMS search
All samples from 2020-2025
Bilirubin and either of coagulation assays sampled at the same time.

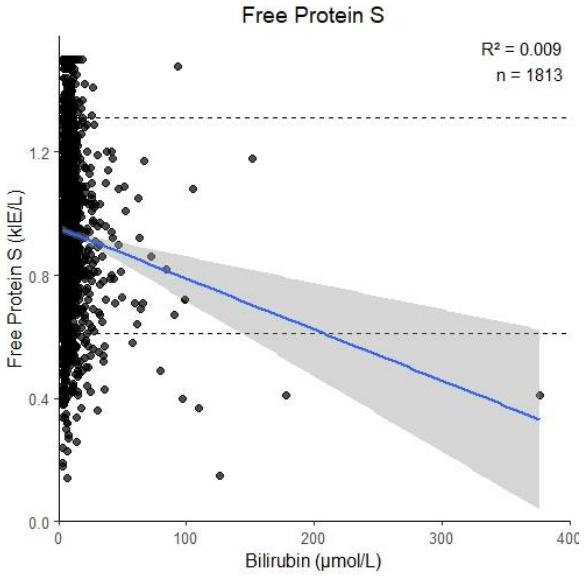
Hypotheses: Free protein S should be unaffacted due to measurment on 800nm. Factor IX should be least affected due to a small plasma volume in the assay. Protein C and FVIII would behave similar in spiking due to have similar plasma volumes in respective assays.

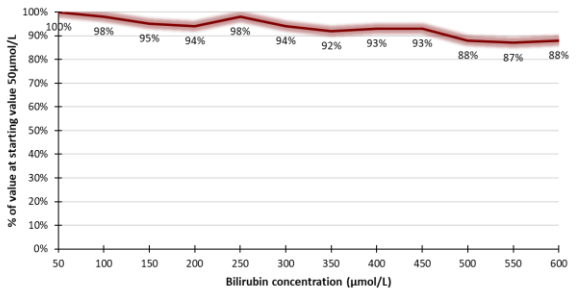
Divergence of results at 405nm detection



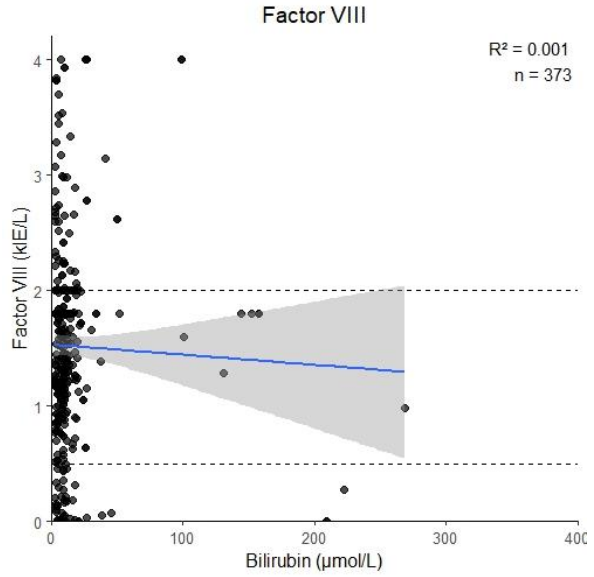
Factor IX, Factor VIII and antitrombin remains stable while Protein C shows a clear drop. This suggest a specific interference of bilirubin with the Berichrom reagent on the Sysmex CS-2500 plattform

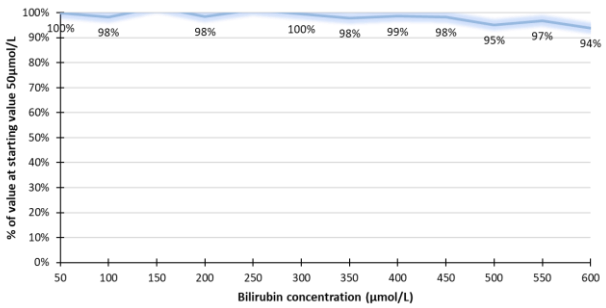
Does hyperbilirubinemia interferens impact clinical interpretations?



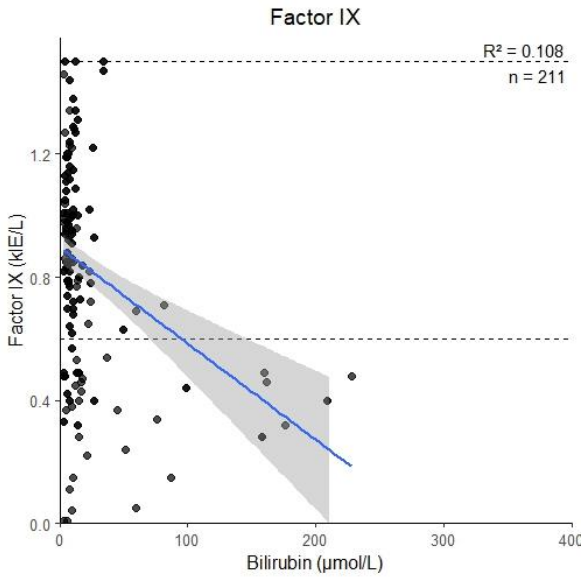


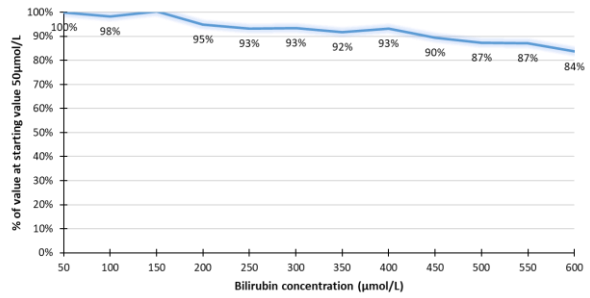
Biological low



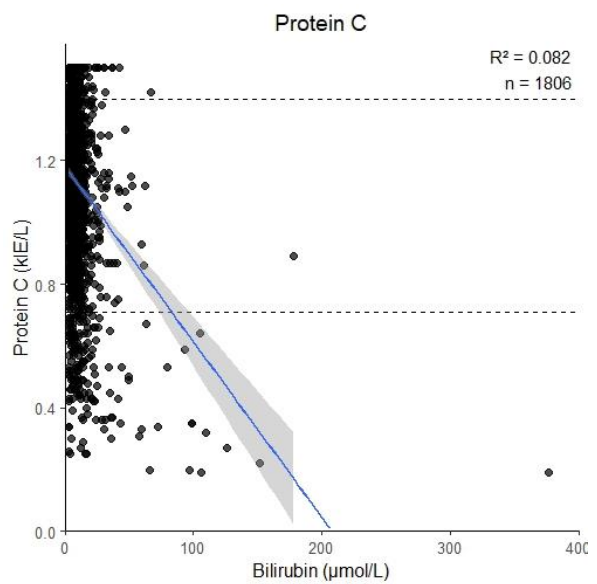


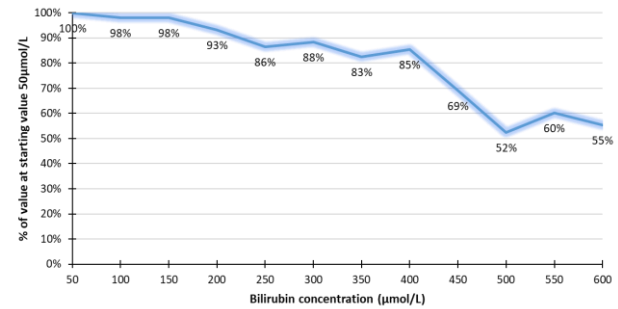
Biological low





Biological low





Biological + analytical low

Conclusions

Non to low degree of analytical interference is seen for VIII, Factor IX and Antithrombin

Analytical interference observed for the Berichrom® Protein C assay on Sysmex CS-2500

The combination of true biological low value and false analytical decrease can give appearance of a more compromised Protein C level

Coagulation laboratories should consider to validate chromogenic assays for icteric patients

Thank you

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