SYDPATH
ST VINCENT'S PATHOLOGY

ST VINCENT'S HOSPITAL
SYDNEY
A FACILITY OF ST VINCENT'S HEALTH AUSTRALIA

Coping with Analytical Interferences

(Handling Icteric, Hemolytic and Lipaemic Samples)

Graham Jones

Department of Chemical Pathology

St Vincent's Hospital, Sydney

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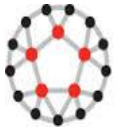
testing for health



RCPAQAP

RCPA Quality Assurance Programs

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Contents

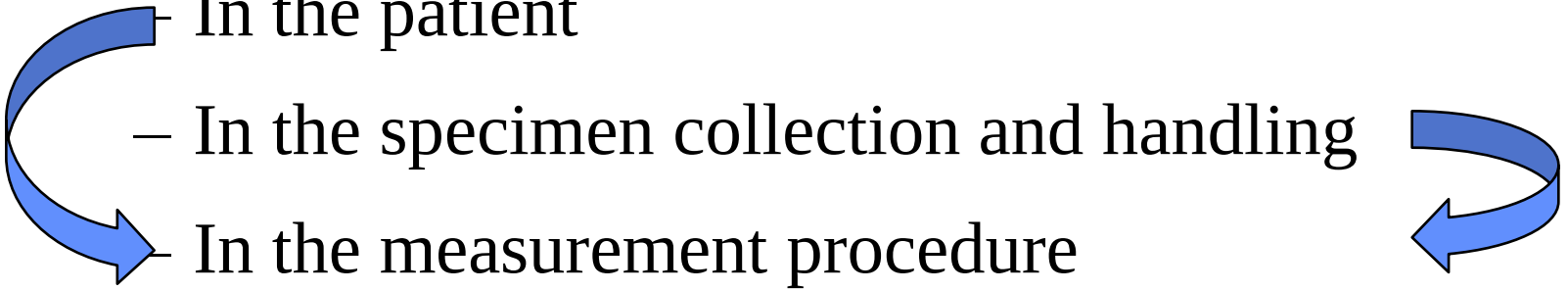
- Background
- Laboratory protocols for common interferences
- Method Evaluation
- Planning to handle common interferences

Background

- Our aim: to produce timely, accurate results to allow optimal patient care
- “Accuracy”: results which reflect the *in-vivo* concentration of an analyte
- Interferents - substances present in a sample, or events affecting a sample, which lead to the production of inaccurate results

Interferences

- May be caused by factors:
  - In the patient
  - In the specimen collection and handling
  - In the measurement procedure
- May affect:
  - All results in collection
  - Some results in collection
  - Only one result from collection



In the patient …

- Icterus
- Lipaemia
- Heterophile antibodies
- “Macro-things” Eg macro prolactin, macro-CK
- Other antibody effects (eg precipitation in phosphate assay)
- Drugs

In the collection…

- Wrong patient
- Wrong label
- Wrong tube
- Drip-arm
- Prolonged tourniquet
- In-vitro haemolysis
- Delayed separation
- Improper handling

In the collection…

- Wrong patient
- W
- D
- P
- In
- D
- In

SOLUTIONS: 1 – In the Collection:

MINIMISE ERROR RATE

Education / training
Resources (correct equipment; information)
Specialist collectors
Monitor and improve

Error Importance

- **Erroneous and Non-believable**
  - eg potassium of 20 mmol/L due to gross haemolysis or EDTA contamination
  - Clinical result: **ignore or recollect specimen**
- **Erroneous and Believable**
  - eg potassium of 5.5 mmol/L due to mild haemolysis
  - Clinical result: **wrongly stop potassium supplements**

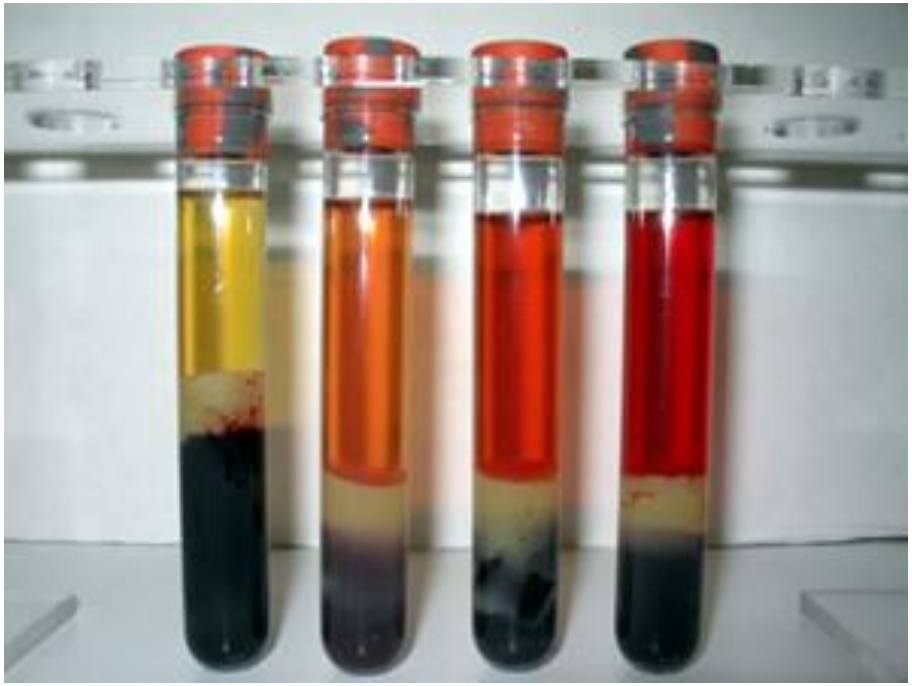
Interferences: Two Types

- **Visible Interferences**
  - Haemolysis, lipemia, icterus
- **Other Interferences**
  - Non-visible interferences (the rest)

Visible Interferents

- Examples of common **visible** interferences.
- Haemolysis, Icterus, Lipaemia
- Detectable by scientists and instruments

In-Vitro Haemolysis

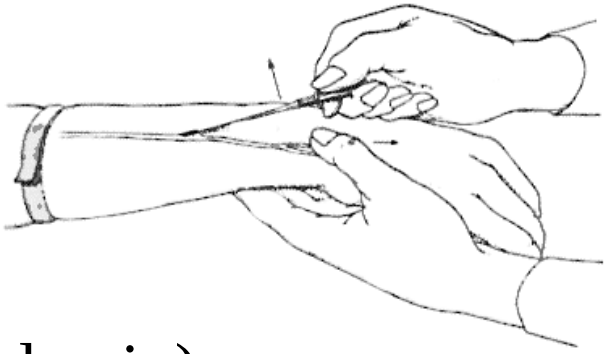


Caused by damage to red cells during collection or handling
Releases red cell contents into serum / plasma

In-Vitro Haemolysis

Most common in the first sample collected in the ED

At time of insertion of cannula.



Collection through plastic, flexible cannula in thin-walled vein
(not firm steel needle in large antecubital vein)

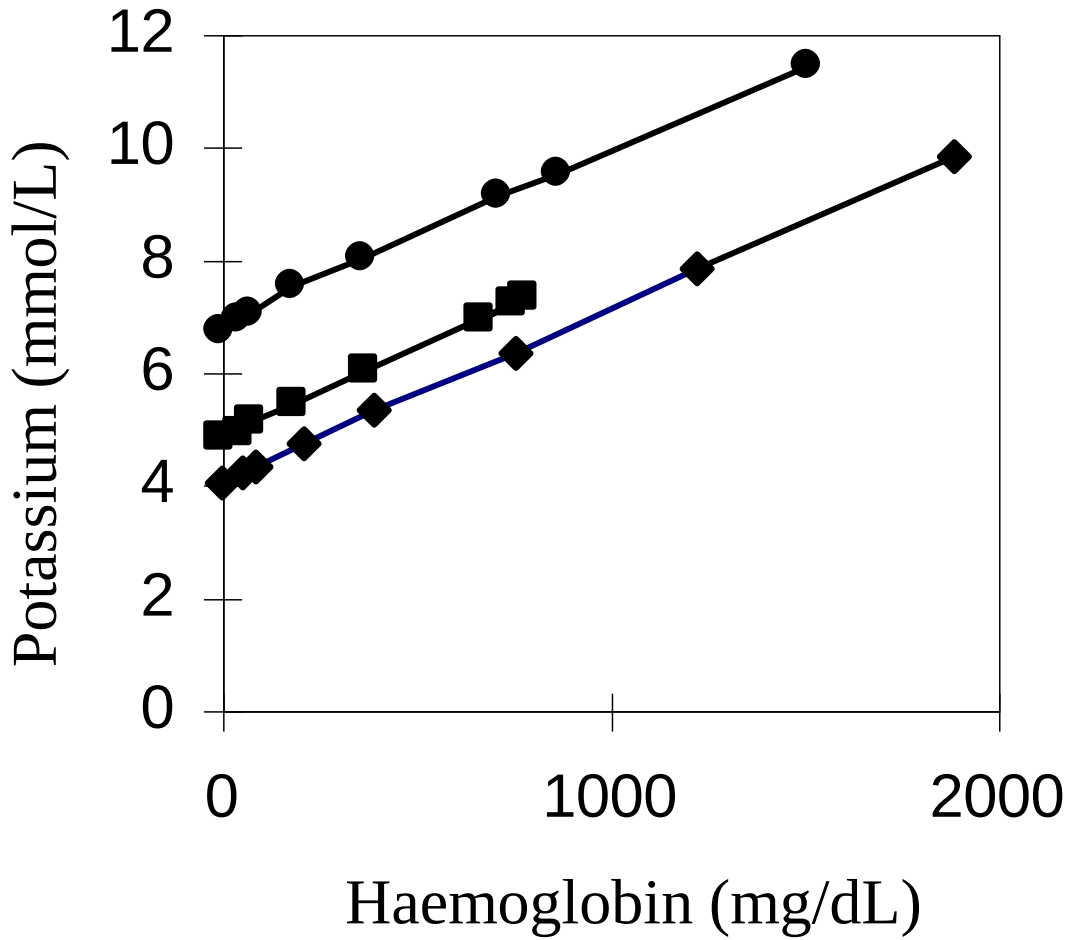
"Sucking" of vessel wall onto cannula → sheer stress
→ in-vitro haemolysis

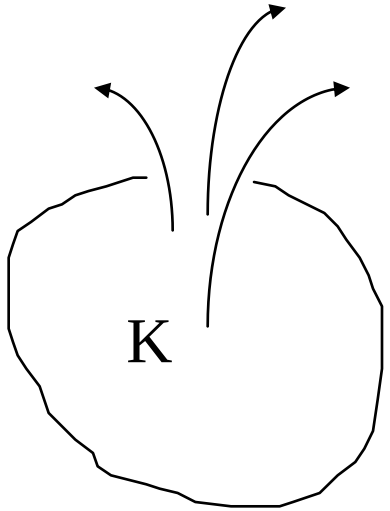
Can affect over 5% of ED samples (fewer from other places)

Also squirting blood through needle into tubes (DO NOT DO)

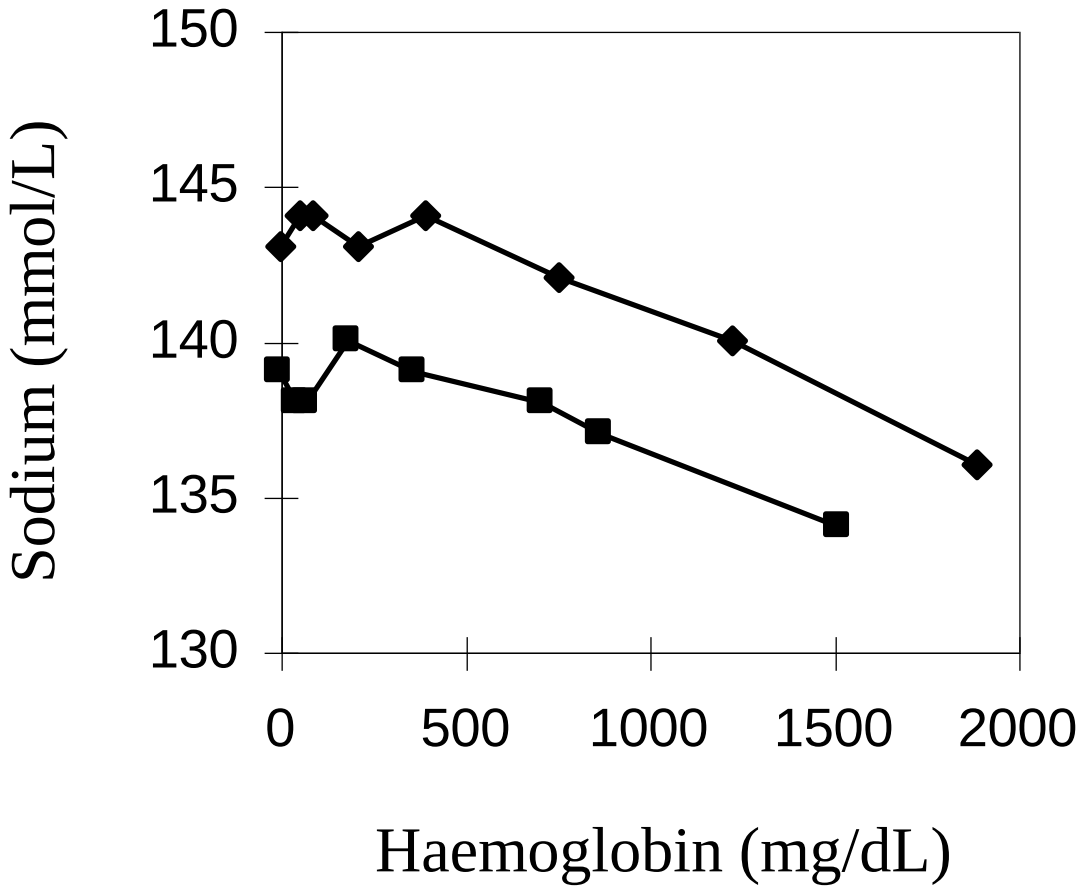
Haemolysis interference - mechanisms

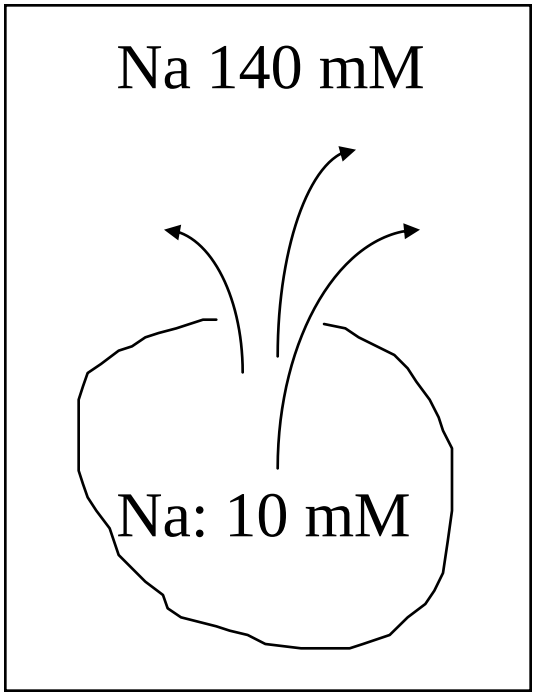
Release of analyte from with red cell



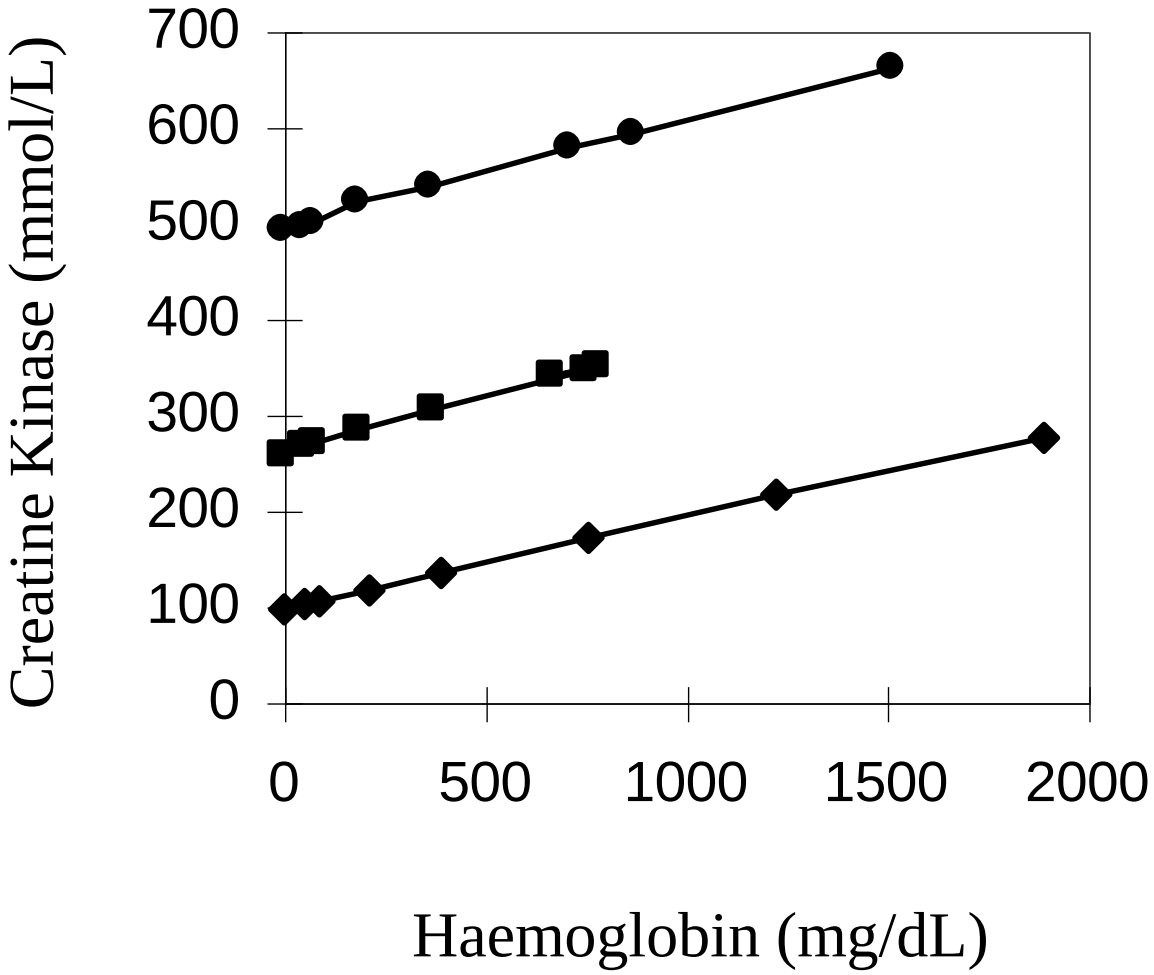


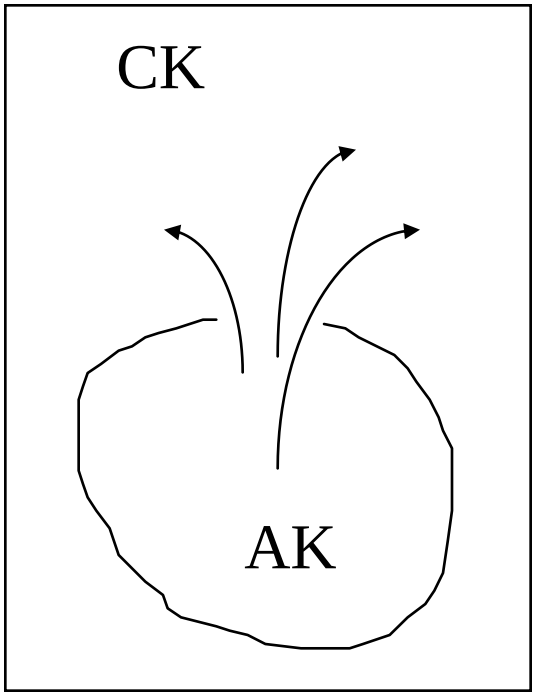
Dilution of analyte: Sodium



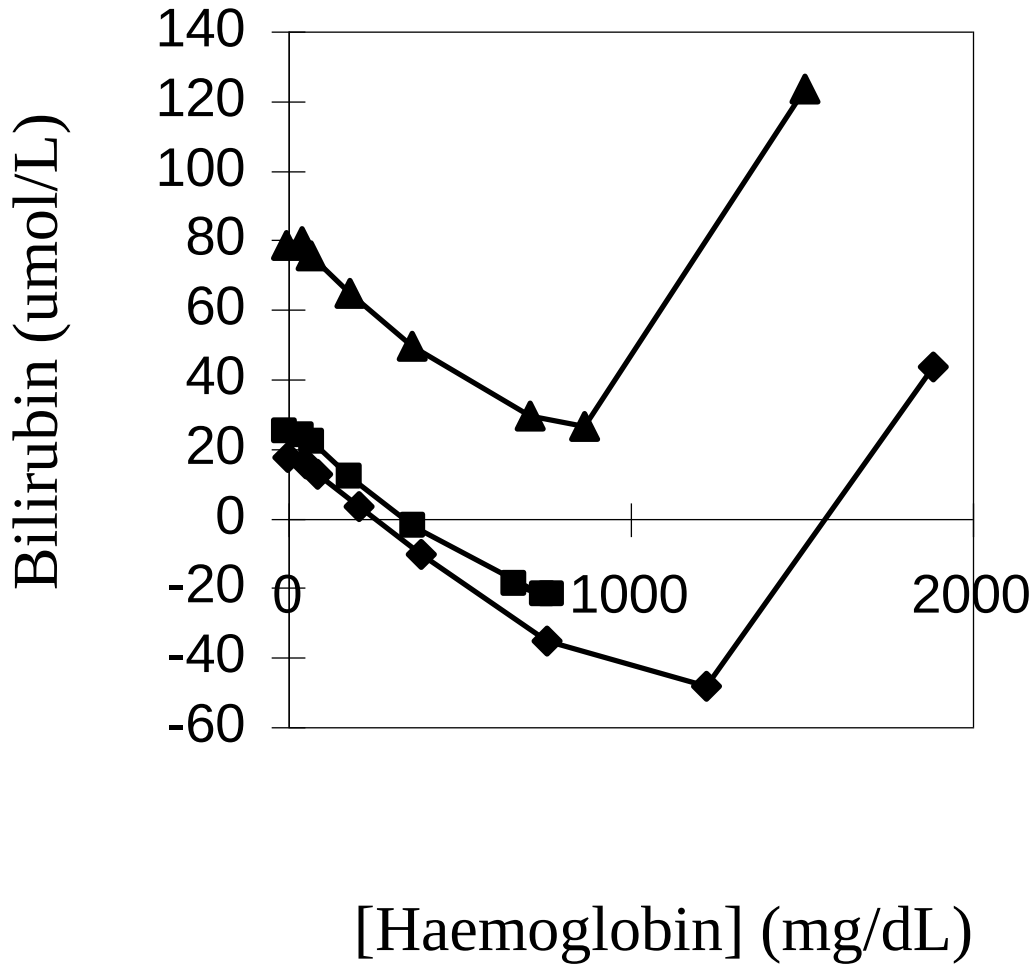


Release of analogue: Creatine Kinase

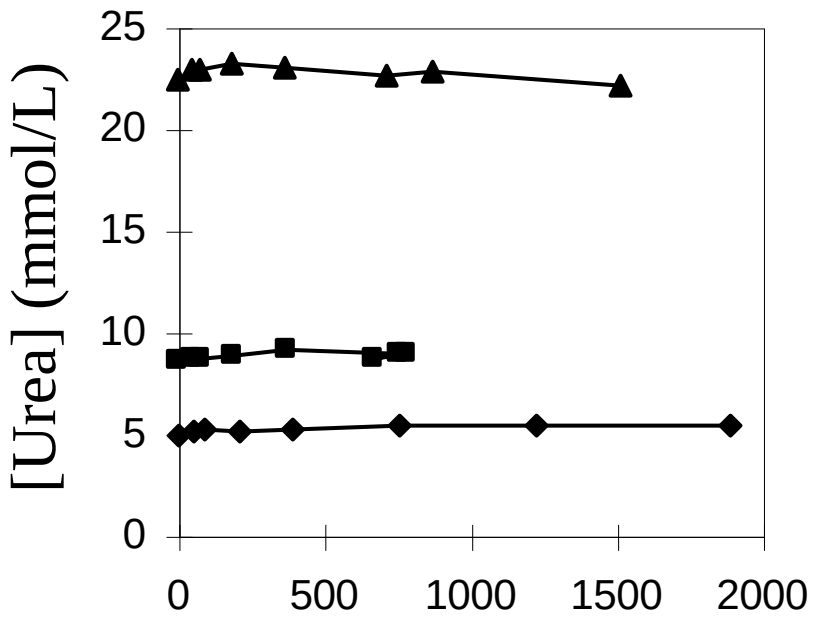


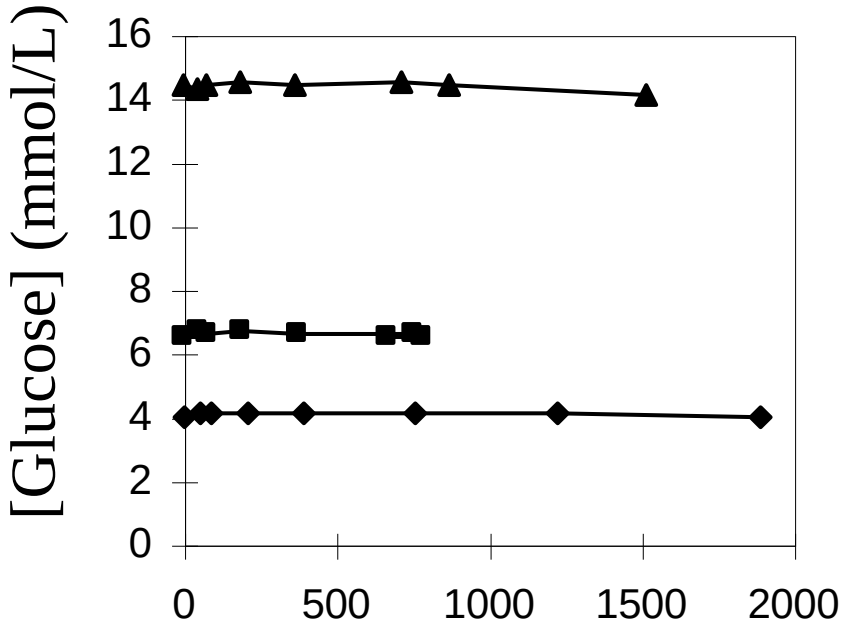


Spectral and Chemical interference: bilirubin



No effects: Urea, Glucose





[Haemoglobin] (mg/dL)

Laboratory Interference Protocols

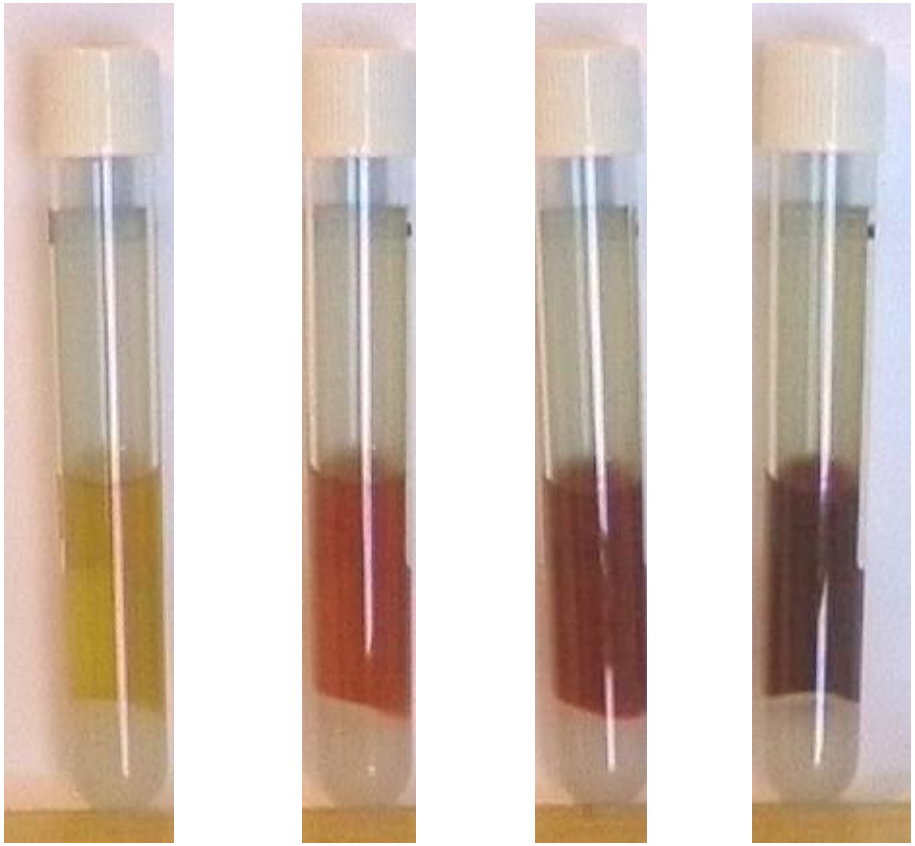
1. Identify presence of interferent
2. Quantify interferent

   Visual, Automated
3. Specify response to interferent

- Lead to reproducible response
- Simple to administer

Identify/Quantify: Visual Interference Chart

HAEMOLYSIS INTERFERENCE CHART



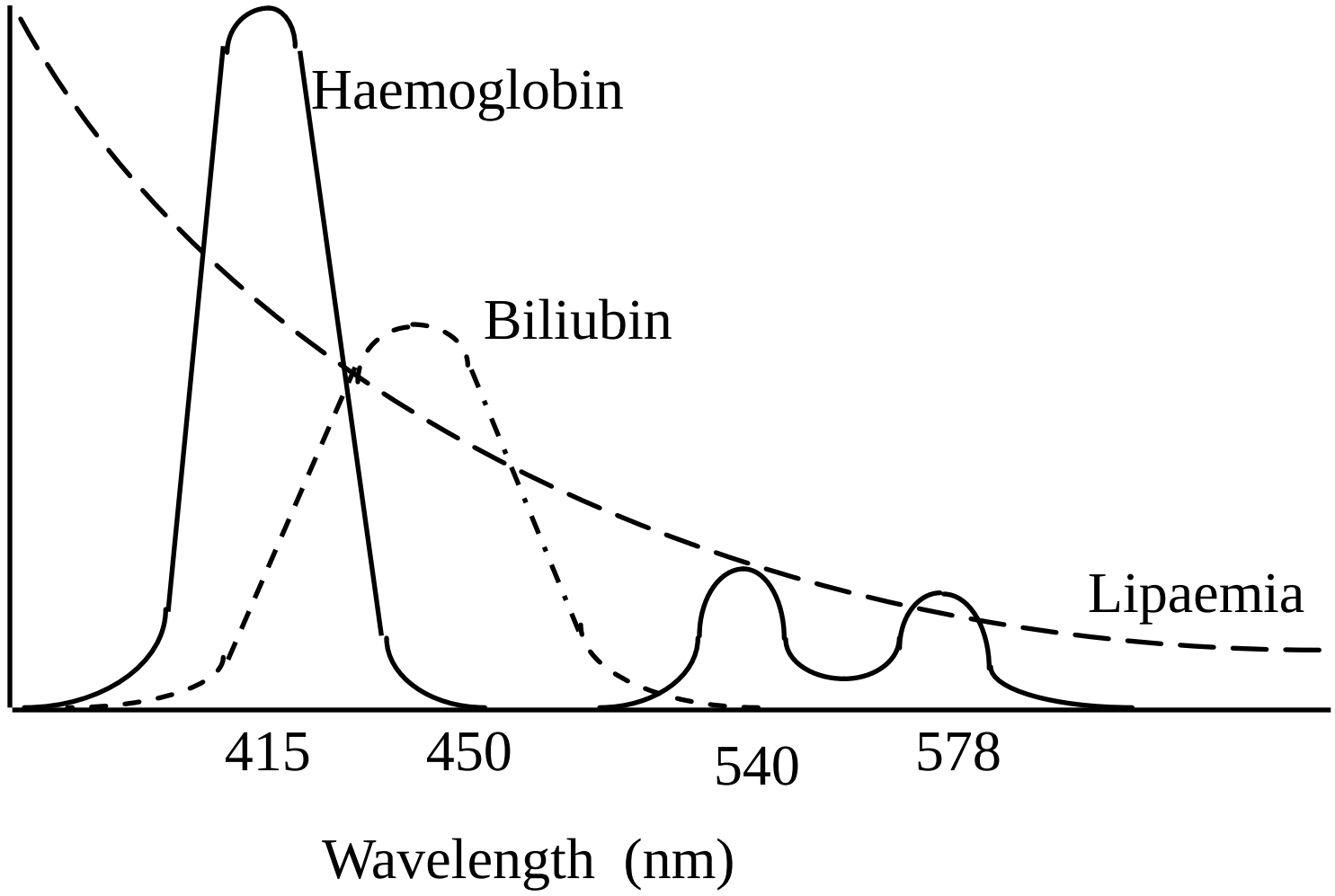
Colour Chart for Comparison of tubes to determine severity of haemolysis.

Haemoglobin expressed in mg/dL.

Instrument Detection

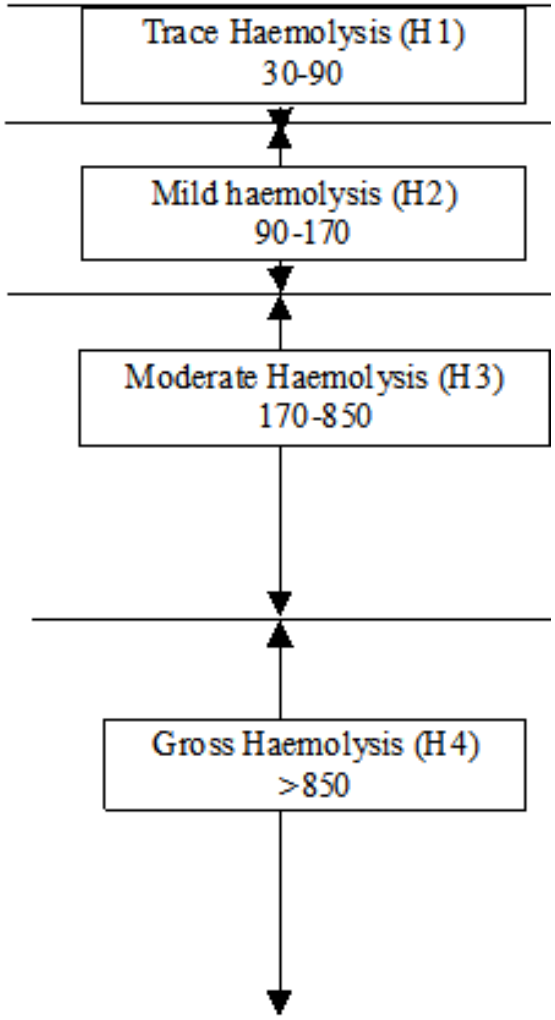
- “Serum Indices”
- Based on absorbance at various wavelengths
- Vary between instruments
- May be quantitative or semi-quantitative
- Often without units
- Instruments
  - Roche, Siemens, Beckman-Coulter, Abbott (chemistry not immunoassay)
  - Others ….?

Haemoglobin, Bilirubin, Lipaemia - spectra



Haemolysis Protocol
(based on serum indices)

Haem (mg/dL)	Analyte
30	LDH
40	LD1
100	Potassium
130	AST, CK
160	Bilirubin, c.Bili
350	Phosphate
500	Fructosamine, Iron
600	Protein
700	Triglycerides
800	Albumin, ALT, GGT, Cholesterol
1000	Amylase, ALP, Bicarbonate, Calcium, CK-MB, Lipase, HDL, Magnesium
1200	Sodium
1500	Uric Acid
2000	Chloride, Creatinine, Glucose, Transferrin, Urea



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Response to Interferents

- Release result with a comment
- With-hold result (with a comment)
- Alternate strategies
  - Recollect (haemolysis only)
  - Remove interferent (lipaemia)
  - Different method (lipaemia and sodium)
  - Correct for effect (haemolysis)

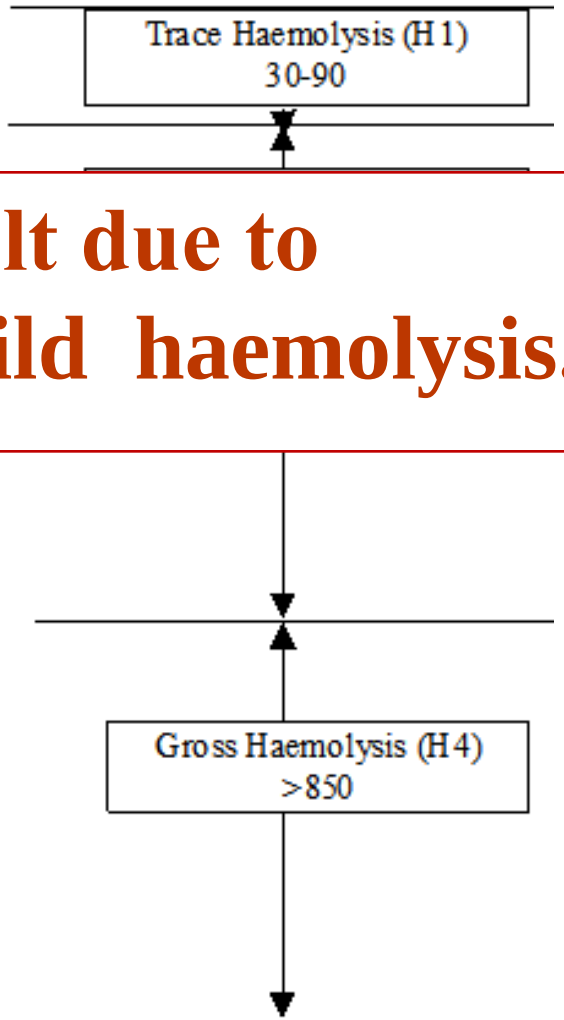
Release with a Comment (examples)

- “Specimen haemolysed”
- “Mildly haemolysed specimen, interpret with caution”
- “Specimen slightly haemolysed. LD rises with haemolysis. Potassium may also increase.”
- “Mildly haemolysed specimen. Results of K, Phosphate, TCO_2, bilirubin, AST, GGT, CK, LDH, Fe and Amylase are affected.”

Haemolysis Protocol
(based on serum indices)

Haem (mg/dL)	Analyte
30	LDH
40	LD1
100	Potassium
700	Triglycerides
800	Albumin, ALT, GGT, Cholesterol
1000	Amylase, ALP, Bicarbonate, Calcium, CK-MB, Lipase, HDL, Magnesium
1200	Sodium
1500	Uric Acid
2000	Chloride, Creatinine, Glucose, Transferrin, Urea

"Potassium: No result due to interference from mild haemolysis."



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Release Corrected results

- More results available, more quickly
- Depends on accuracy of correction
- Fewer recollections
- SydPath: Potassium, CK, Troponin T *(not widely done!)*

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Correcting laboratory results for the effects of interferences: an approach incorporating uncertainty of measurement

Annals of Clinical Biochemistry 2015;52:226-31

Graham RD Jones[1] and Robert C Hawkins[2]

Interference Planning

- (choice of instrument / reagents)

- Evaluate current instrument / reagents
- Define Allowable Error
- Set limits for interferents
- Define response to excessive interference

Evaluate Instruments / Methods

- Source material
  - Manufacturers claims*
  - Published evaluations
  - Local Studies

- *Manufacturers claims
  - Review criteria for “no effect”
  - May be limited in testing range

Manufacturer - Example

Limitations – interference[12,15]

For determination of cholesterol with lipase on Roche/Hitachi 704/717 analyzers, refer to the R1/R2 application in the Instrument Settings section.

US users: Refer to application sheet.

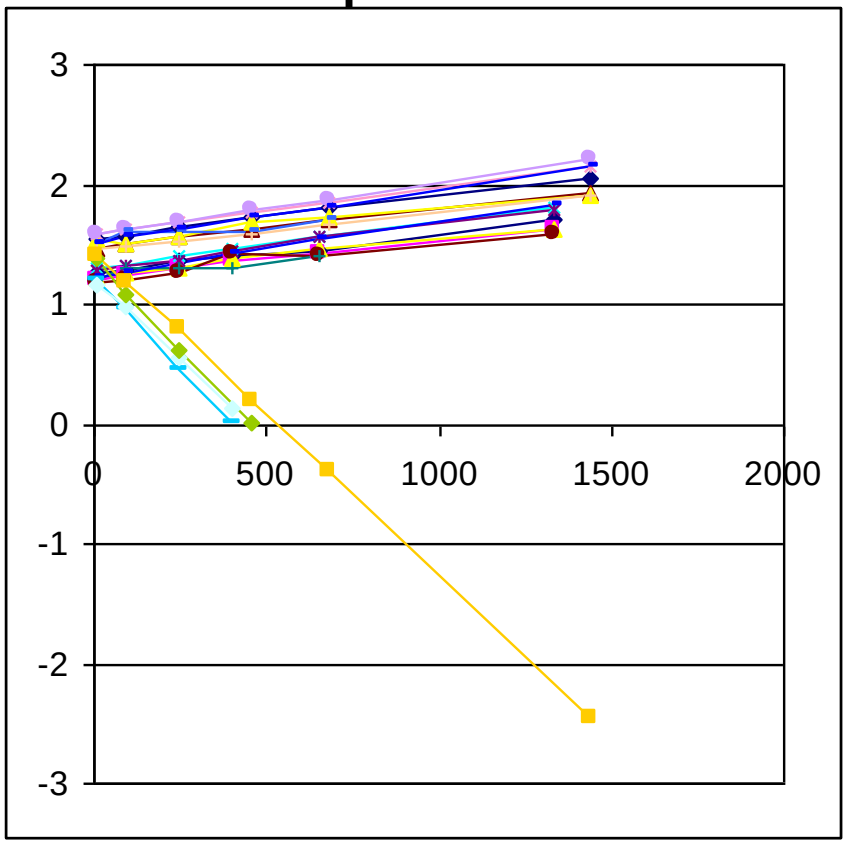
Criterion: Recovery within ± 10% of initial values.

Icterus: No significant interference up to an I index of 25 (approximate conjugated bilirubin concentration: 25 mg/dl or 428 µmol/l) and an I index of 10 (approximate unconjugated bilirubin concentration: 10 mg/dl or 171 µmol/l).

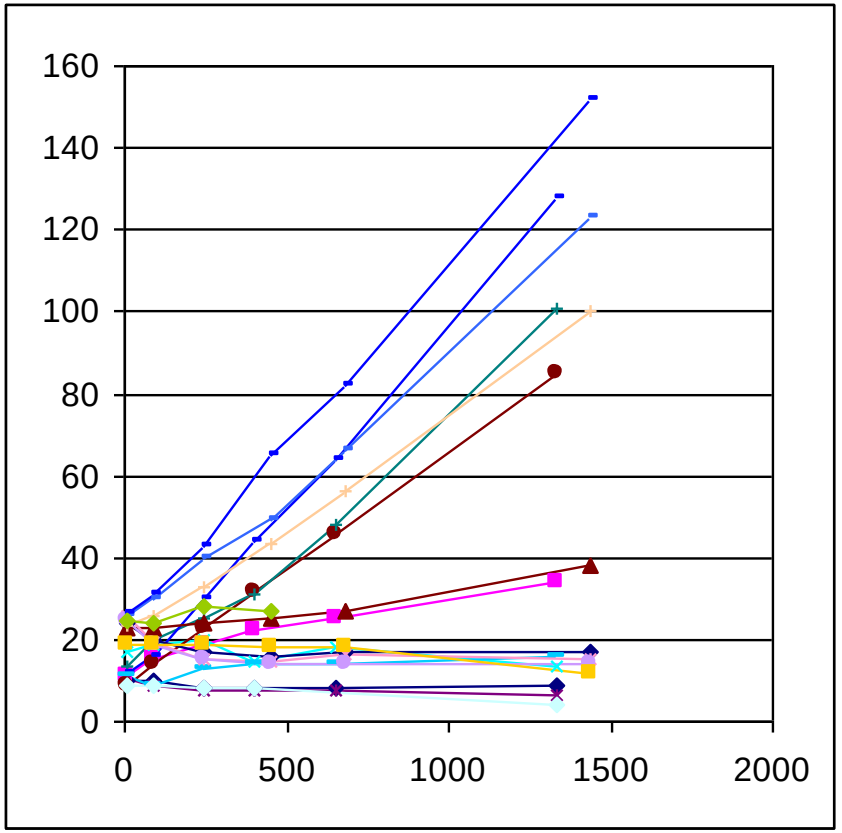
Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 700 mg/dl or 435 µmol/l).

Method differences

Phosphate



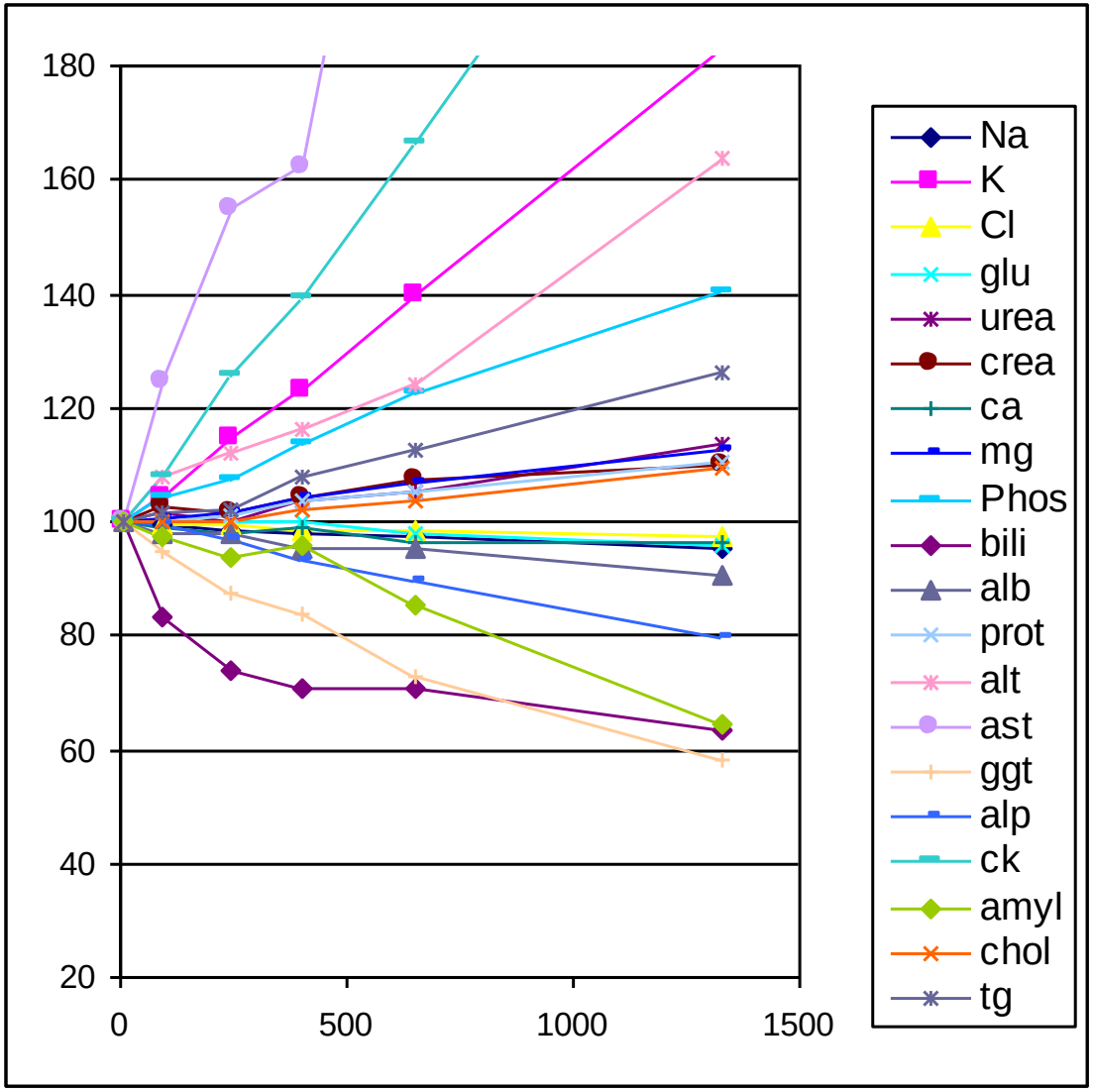
Bilirubin



Haemoglobin (mg/dL)

Haemolysis effect for 7 methods on 2 analytes.
NOT ALL METHODS THE SAME.

Interferogram



- Haemolysis Effect
- Roche Modular P
- Haemolysate added to sample
- Percent measured plotted against haemoglobin

- (Melvin Glick)

Define Allowable Error

- RCPA-AACB Quality Assurance limits
- Change greater than 2 SD of analytical precision
- Change related to biological variation
- 10%
- Other fixed percentage or absolute values
- A difference that may lead to a change in clinical management - subjective*

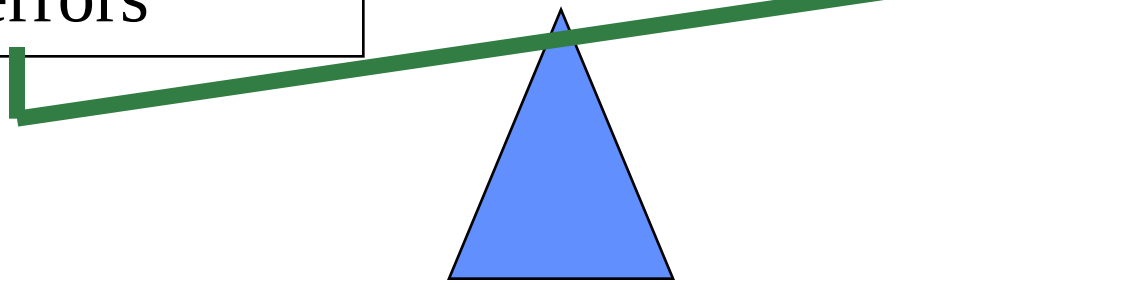
The Accuracy - Utility Balance

Tighter Limits

More accuracy
More rejections
More recollections
More delays
Unhappier ptns and Drs
Fewer clinical errors

Looser Limits

Less accuracy
Fewer rejections
Fewer recollections
Shorter TAT
More clinical errors

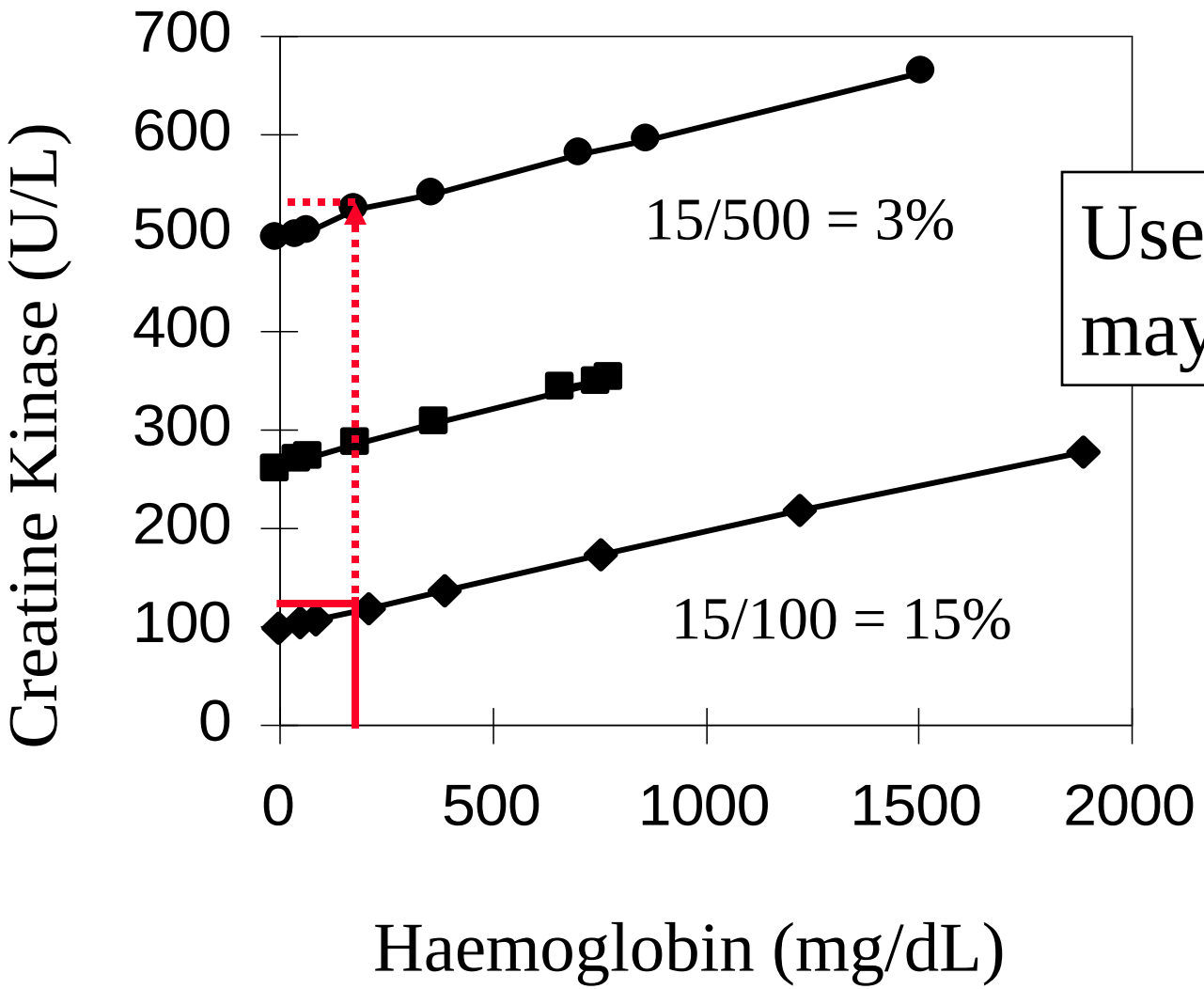


Other factors

- Intravascular haemolysis
- With-holding potentially useful results
- Releasing erroneous results

- Effect of concentration

Absolute Limits



Useful results may be with-held

Summary

- Interferences affect our core function in rou
- Lab ide hae
- Pla ins your clients.

SOLUTIONS: 2 – Visible interferences:

LABORATORY PROTOCOL

Identify
Quantify
Respond
- with-hold, comment, correct, other

Non-Visible Interferences

Non-Visible Interferences

- Wrong patient, Wrong tube, Aliquotting error
- Drip-arm, Prolonged tourniquet
- Delayed separation, Improper handling
- Heterophile antibodies
- “Macro-things” Eg macro prolactin
- Other antibody effects (eg precipitation in phosphate assay)
- Drugs

Non-Visible Interferences

- Not apparent until results obtained
- May see:
  - Extreme results
  - Patterns: eg ETDA, drip arm, delayed separation
  - Changes since last measurement (delta)

Non-Visible Interferences

- Only tool: Results Review
- I
- I
- E
- F
- Communicate with requester

SOLUTION 3 – Non-visible interferences:

LABORATORY PROTOCOL

Result Review
Identify likely cause
Respond
- check results, recollect, d/w team

Summary

- Interferences affect our core function in routine pathology laboratories.
- Laboratories must have protocols for:
  - Collecting samples correctly
  - Identifying, quantifying and responding to "coloured" samples.
  - Identifying, evaluating and responding to unusal patters of results.
- Best practice involves communication of processes and results with clinicians

End

Handling Common Interferences.

Graham Jones

Clin Biochem Revs, 2002;23:101-128.