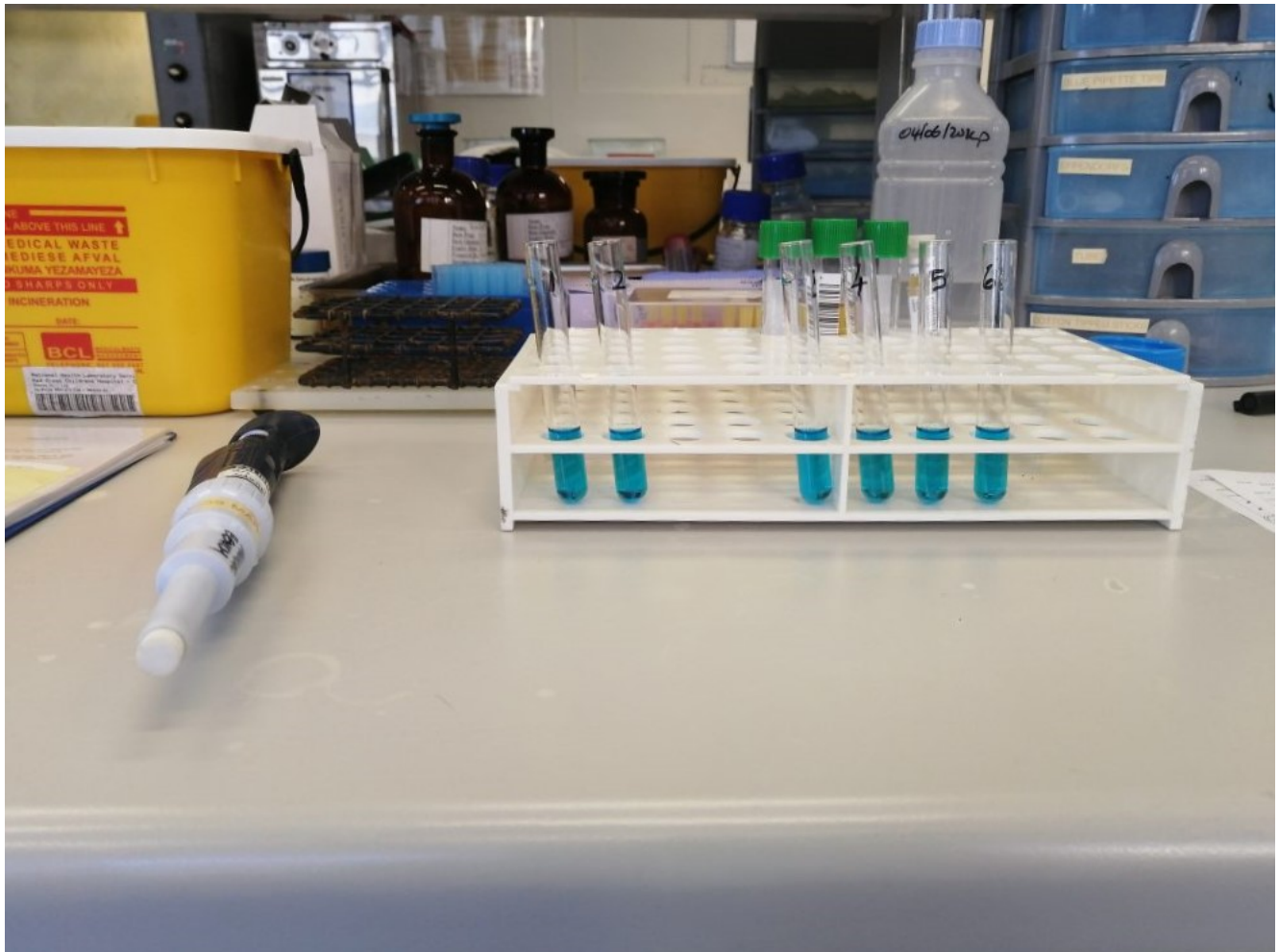


# Screening for reducing substances using Benedict's reagent



## Abstract

Disorders of carbohydrate metabolism result in the accumulation of reducing substances. These are often present in the urine and stool of affected individuals and are not present in unaffected individuals. A screening test using Benedict's reagent can be a quick and simple way to identify these substances. A colour change from blue to brick red is a positive result. Definitive testing can then be carried out.

# Introduction

Reducing substances comprise all the sugars exhibiting ketonic. (2) A commonly used qualitative method for screening employs Benedict's reagent. In this reaction, cupric ion ( $\text{Cu}^{3+}$ ) is complexed to citrate in an alkaline solution. The reducing substances convert cupric ( $3+$ ) to cuprous ( $2+$ ) ions. This results in a colour change with the formation of yellow cuprous hydroxide or red cuprous oxide. (1) Sugars (e.g, glucose, galactose, fructose, maltose, lactose, and pentose) are characterized as reducing substances based on their ability to reduce cupric ions to cuprous ions. Reducing substances are often requested when carbohydrate malabsorption is suspected. (3)

## BOX 33.2 Reducing Substances in Urine

|               |                   |
|---------------|-------------------|
| Fructose      | Ketone bodies     |
| Lactose       | Sulfanilamide     |
| Galactose     | Oxalic acid       |
| Maltose       | Hippuric acid     |
| Arabinose     | Homogentisic acid |
| Xylose        | Glucuronic acid   |
| Ribose        | Formaldehyde      |
| Uric acid     | Isoniazid         |
| Ascorbic acid | Salicylates       |
| Creatinine    | Cinchophen        |
| Cysteine      | Salicyluric acid  |
| Glucose       |                   |

Examples of reducing substances found in urine. Taken from *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 6th edition pages

# Methods

## Materials:

Controls: Positive and negative

Patient samples (urine and stool)

Benedict's reagent

## Equipment:

Heating block

Thermometers

Metal cap

Centrifuge

Vortex

Consumables

## Control material

The positive control is made by dissolving 1g/50mL D-galactose into deionized water. Deionized water is used as a negative control.

## Reagent

Benedict's reagent was already pre-made for this experiment.

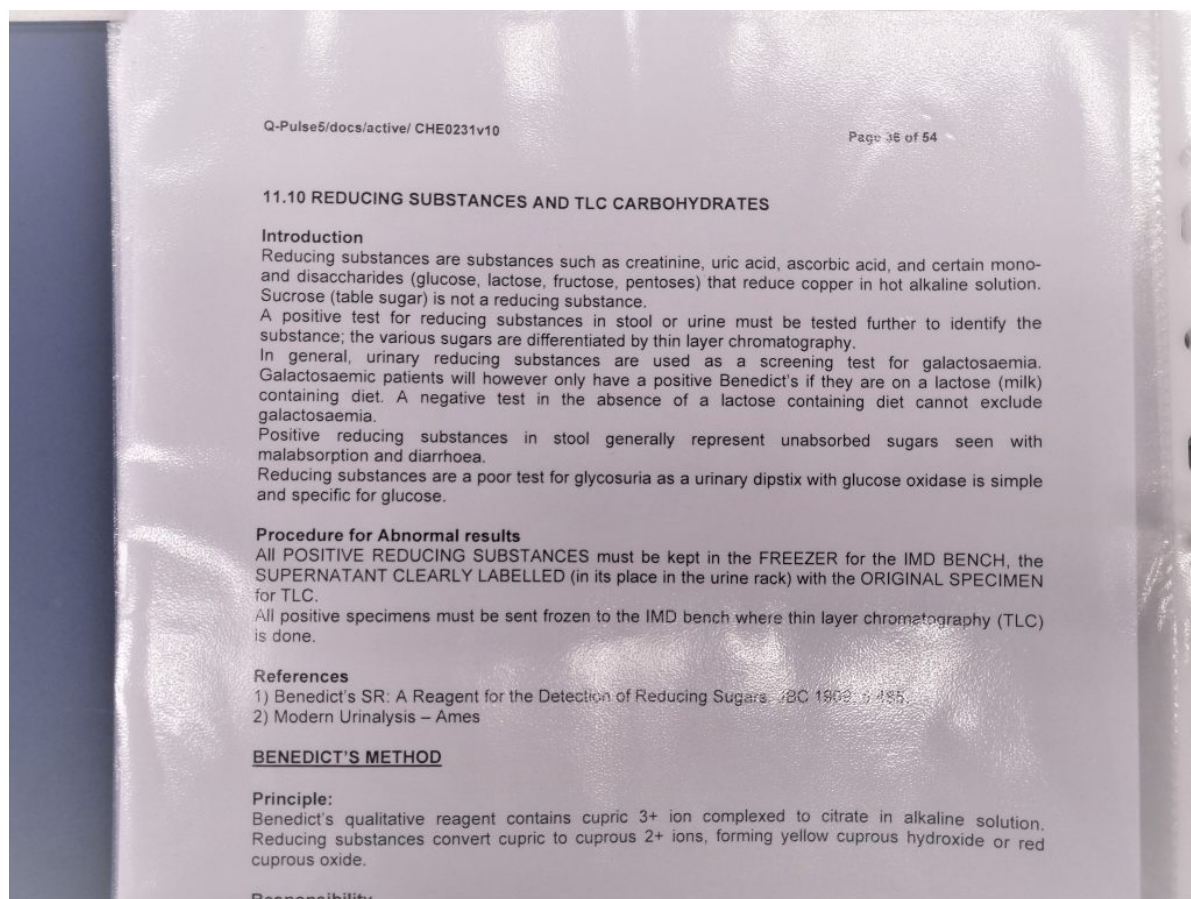
Method to prepare reagent is as follows: 17.3g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100mL hot water. In a separate container, 173g of  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 7.2\text{H}_2\text{O}$  and 100g  $\text{Na}_2\text{CO}_3$  in 800mL deionised water, heat solution. Once cool, both solutions are mixed and diluted up to a total volume of 1L.

## Sample preparation

Urine samples: Samples that were not clear were centrifuged at 4000rpm for 5 minutes.

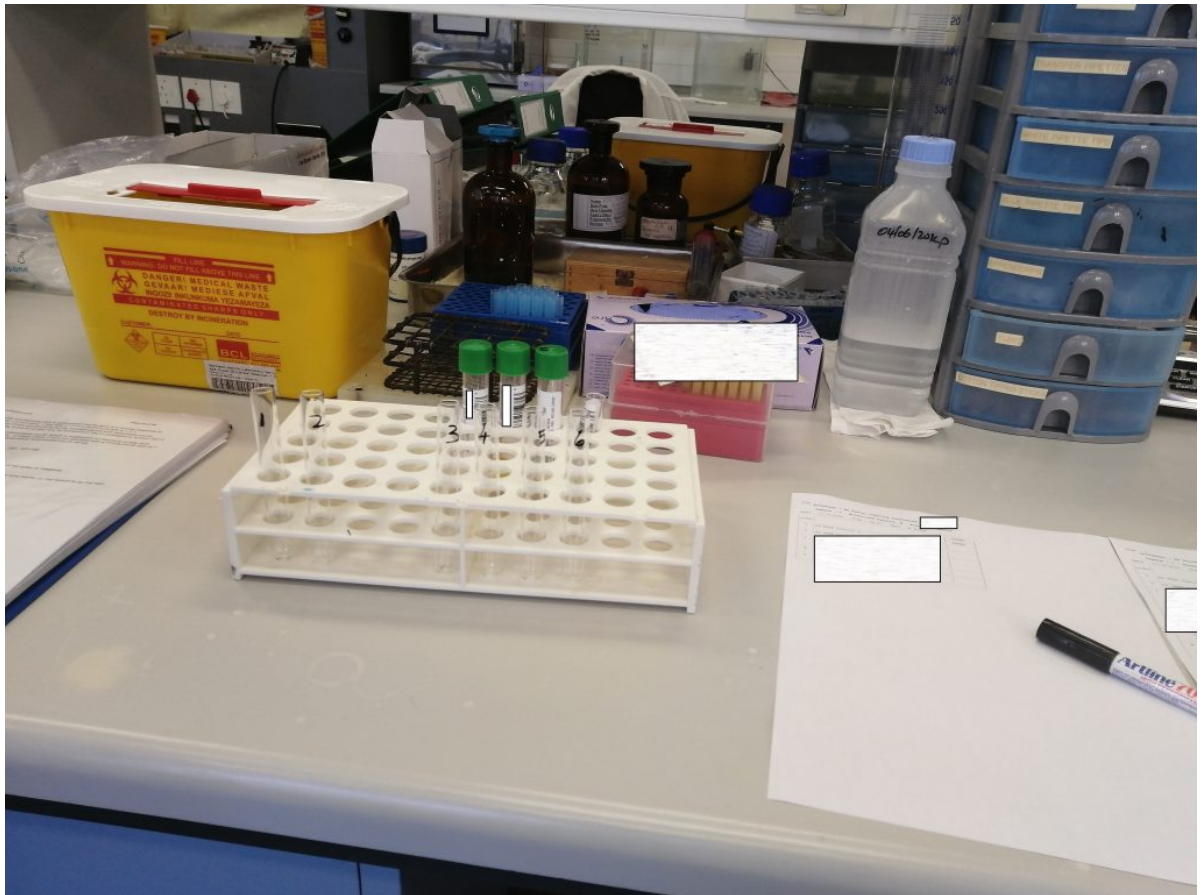
Stool sample: only 1 stool sample was received and had a loose consistency. About 1.5mL of the sample was centrifuged at 4000rpm for 5 minutes.

2.mL of Benedict's reagent was added to all of the test tubes. 5 drops of sample was added to each test tube. Tubes covered with a metal cap and vortexed. The samples were placed in a beaker of water at 100degrees Celsius for 3 minutes, while the water was still boiling. Samples were then vortexed again and allowed to sit for 10 minutes before reading.

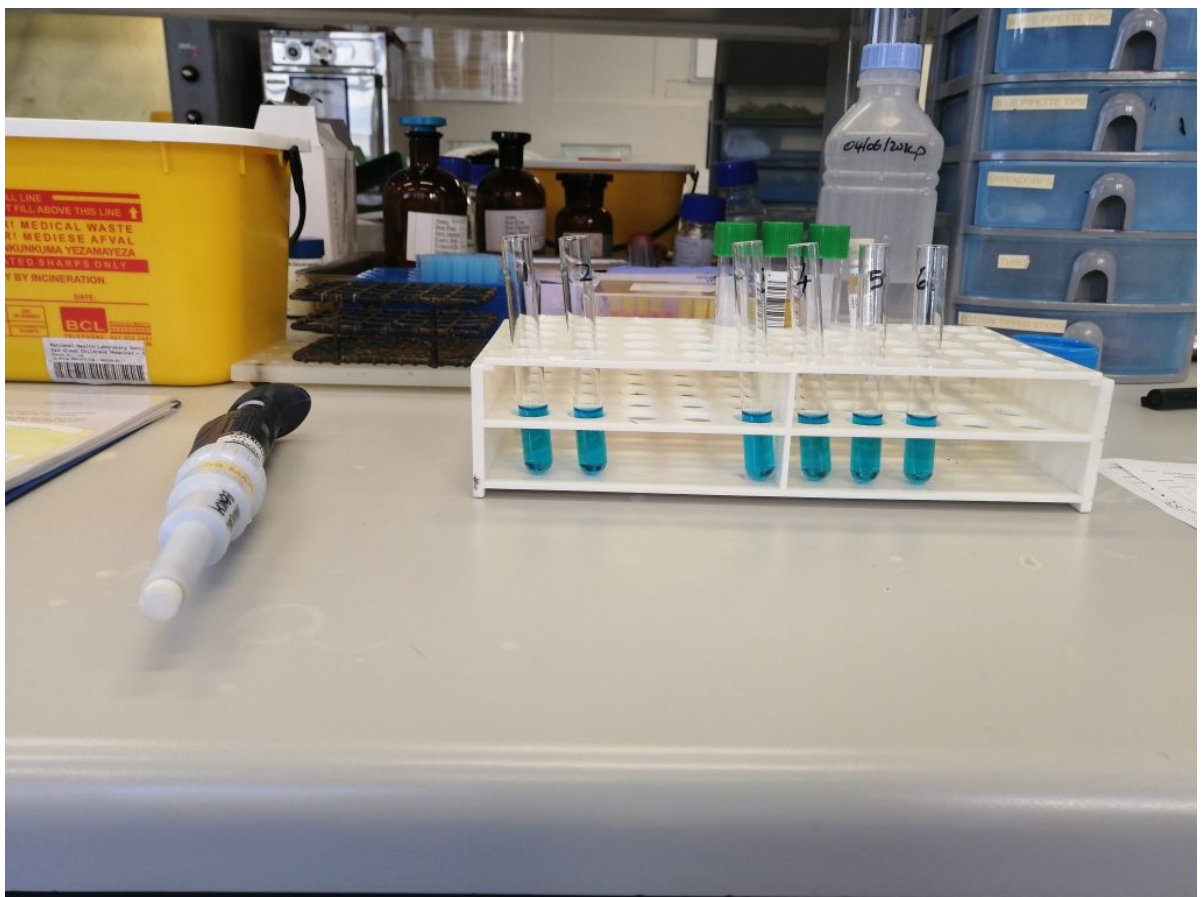


Standard operating procedure used for this method

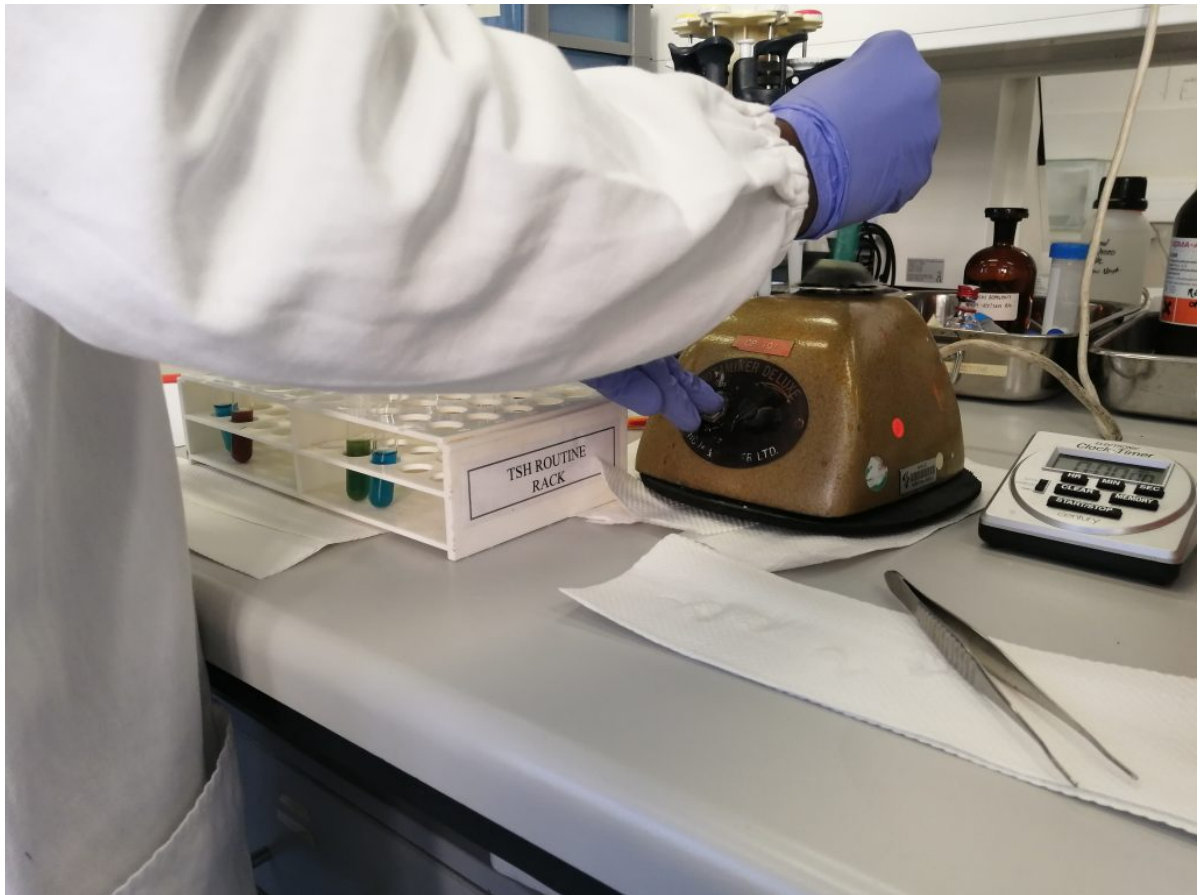




Setting up



2.5 mL Benedict's reagent with no sample



Samples vortexed after being added to the reagent.



Incubate for 3 minutes in continuously boiling water at



100 degrees Celsius.

Tests that screen positive are then analyzed using more sensitive techniques. In our laboratory, this is with thin layer chromatography (TLC).

## Results

The control samples exhibited the colour changes that are to be anticipated. The negative control does not form cuprous ions (which are red) thus no colour change occurs. The galactose in the positive control acts as a reducing substance and converts cupric (3+) to cuprous (2+) ions (thus resulting in the brick-red colour). The patient samples exhibited a trace result with a mild change to a green colour, likely due to the formation of yellow cuprous hydroxide. The 1+ positive sample had yellow precipitate with sedimentation visible in the bottom and top layer of the solution.



Negative control on left, positive control on right.



Trace positive on left, negative control on right.



Negative control on left, 1+ positive on right (note



yellow precipitate)

## Discussion

False-positive results may be produced by contamination of urine with H<sub>2</sub>O<sub>2</sub> or a strong oxidizing agent, such as hypochlorite (bleach). Exposure of dipsticks to air gives false-positive readings after 7 days. False-negative results may occur with large quantities of reducing substances, such as ketones, ascorbic acid, and salicylates. Several antibiotics that contain ascorbic acid as a preservative may also produce false-positive results as it is excreted essentially unchanged.

Clinically, a negative test in the absence of a lactose containing diet cannot exclude galactosaemia.

Reducing substances other than glucose should be further identified by chromatographic procedures. The measurement of glucose has now been replaced with more-specific, convenient and inexpensive methods.

## References

1. Tietz Textbook of Clinical Chemistry,
  2. Compendium of International Methods of Analysis- OIV: Reducing Substances
  3. Faecal Reducing Substances.  
<https://www.gloshospitals.nhs.uk/our-services/services-we-offer/pathology/tests-and-investigations/faecal-reducing-substances/>
-

## 23. Urine sodium

|            |            |           |            |
|------------|------------|-----------|------------|
| Ward       | Not stated | D.O.B/Age | 10/06/1958 |
| Consultant |            |           |            |

Urine sodium 224 mmol/L

Request form: hyponatraemia

Many patients present due to manifestations of other medical comorbidities, with hyponatremia being recognized only secondarily. Many medical illnesses, such as chronic heart failure, liver failure, renal failure, or pneumonia, may be associated with hyponatremia. Patients usually present with symptoms related to their primary illness.

Symptoms of hyponatremia range from nausea and malaise, with a mild reduction in the serum sodium, to lethargy, decreased level of consciousness, headache, and (if severe) seizures and coma. Overt neurologic symptoms most often are due to very low serum sodium levels (usually < 115 mmol/L), resulting in intracerebral osmotic fluid shifts and brain oedema.

Examination should include orthostatic vital signs and an accurate assessment of volume status. Volume status forms an integral part of assessment as it often guides assessment and treatment.

A full assessment for medical comorbidities is also essential, with particular attention to cardiopulmonary and neurologic components of the examination.

Authorised by Dr TA Gcingca on 05/01/2020 at 21:37  
Urine sodium 224 mmol/L

Authorised by Dr TA Gcingca on 05/01/2020 at 21:37  
Urine osmolality 649 mmol/kg 50 - 1200

CT brain and CXR may be indicated if SIADH suspected.

True hyponatraemia.

Hyponatremia can be classified according to volume status, as follows:

- Hypovolemic hyponatremia: decrease in total body water with greater decrease in total body sodium
- Euvolemic hyponatremia: normal body sodium with increase in total body water
- Hypervolemic hyponatremia: increase in total body sodium with greater increase in total body water

Hyponatremia can be further subclassified according to effective osmolality, as follows:

- Hypotonic hyponatremia
- Isotonic hyponatremia
- Hypertonic hyponatremia

There are three essential laboratory tests in the evaluation of patients with hyponatremia that, together with the history and the physical examination, help to establish the primary underlying etiologic mechanism: urine osmolality, serum osmolality, and urinary sodium concentration.

1. Urine osmolality: essential to differentiate a deficiency in excreting free water vs primary polydipsia. Urine osmolality greater than 100 mOsm/kg



indicates impaired ability of the kidneys to dilute the urine.

2. Serum osmolality: differentiates between true hyponatremia and pseudohyponatremia. True hyponatraemia causes an decrease in serum osmolality.
3. Urinary sodium: helps to differentiate between hyponatremia secondary to hypovolemia and the syndrome of inappropriate ADH secretion (SIADH). With SIADH (and salt-wasting syndrome), the urine sodium is greater than 20-40 mmol/L. With hypovolemia, the urine sodium typically measures less than 25 mmol/L.

Ancillary testing may also help with differentiating SIADH from salt-wasting. Serum uric acid levels can be important supportive information (they are typically reduced in SIADH and also reduced in salt wasting). After correction of hyponatremia, the hypouricemia corrects in SIADH but remains with a salt-wasting process.

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## 7. EDTA contamination vs renal impairment

|            |              |           |            |
|------------|--------------|-----------|------------|
| Ward       | Surgical ICU | D.O.B/Age | 17/04/1994 |
| Consultant |              |           |            |

Potassium: 6.1 H mmol/L [3.5 – 5.1]

No diagnosis on request form, unable to get hold of clinician.

Authorised by Dr TA Gcingca on 27/11/2019 at 08:37

|        |     |        |           |
|--------|-----|--------|-----------|
| Sodium | 137 | mmol/L | 136 - 145 |
|--------|-----|--------|-----------|

Authorised by Dr TA Gcingca on 27/11/2019 at 08:37

|           |       |        |           |
|-----------|-------|--------|-----------|
| Potassium | 6.1 H | mmol/L | 3.5 - 5.1 |
|-----------|-------|--------|-----------|

Authorised by Instrument on 27/11/2019 at 06:11

|          |     |        |          |
|----------|-----|--------|----------|
| Chloride | 106 | mmol/L | 98 - 107 |
|----------|-----|--------|----------|

Authorised by Dr TA Gcingca on 27/11/2019 at 08:37

|      |        |        |           |
|------|--------|--------|-----------|
| Urea | 19.7 H | mmol/L | 2.1 - 7.1 |
|------|--------|--------|-----------|

---

Authorised by Instrument on 27/11/2019 at 06:11

|                     |       |                            |          |
|---------------------|-------|----------------------------|----------|
| Creatinine          | 198 H | umol/L                     | 64 - 104 |
| eGFR (MDRD formula) | 38    | mL/min/1.73 m <sup>2</sup> |          |

MDRD-derived estimation of GFR may significantly underestimate true GFR in patients with GFR > 60 mL/min/1.73m<sup>2</sup>. It may also be unreliable in the case of: age <18 years or >70 years; pregnancy; serious co-morbid conditions; acute renal failure; extremes of body habitus/unusual diet; gross oedema. The MDRD-eGFR used here does not employ an ethnic factor for race.

Authorised by Dr TA Gcingca on 27/11/2019 at 08:37

|         |        |        |             |
|---------|--------|--------|-------------|
| Calcium | 1.17 L | mmol/L | 2.15 - 2.50 |
|---------|--------|--------|-------------|

Authorised by Dr TA Gcingca on 27/11/2019 at 08:37

|           |      |        |             |
|-----------|------|--------|-------------|
| Magnesium | 0.97 | mmol/L | 0.63 - 1.05 |
|-----------|------|--------|-------------|

Authorised by Instrument on 27/11/2019 at 06:11

|                     |        |        |             |
|---------------------|--------|--------|-------------|
| Inorganic phosphate | 1.46 H | mmol/L | 0.78 - 1.42 |
|---------------------|--------|--------|-------------|

Authorised by Instrument on 27/11/2019 at 06:11

Indices in serum:

|                   |              |
|-------------------|--------------|
| Haemoglobin index | Not detected |
| Bilirubin index   | Trace        |
| Lipaemia index    | Not detected |

Authorised by Instrument on 27/11/2019 at 05:44

|                             |         |                       |               |
|-----------------------------|---------|-----------------------|---------------|
| White Cell Count            | 10.17   | x 10 <sup>9</sup> /L  | 3.92 - 10.40  |
| Red Cell Count              | 3.32 L  | x 10 <sup>12</sup> /L | 4.50 - 5.50   |
| Haemoglobin                 | 9.8 L   | g/dL                  | 13.0 - 17.0   |
| Haematocrit                 | 0.274 L | L/L                   | 0.400 - 0.500 |
| MCV                         | 82.5 L  | fL                    | 83.1 - 101.6  |
| MCH                         | 29.5    | pg                    | 27.8 - 34.8   |
| MCHC                        | 35.8 H  | g/dL                  | 33.0 - 35.0   |
| Red Cell Distribution Width | 15.2    | %                     | 12.1 - 16.3   |
| Platelet Count              | 116 L   | x 10 <sup>9</sup> /L  | 171 - 388     |

Potassium ethylenediaminetetraacetic acid (EDTA) is a sample tube anticoagulant used for many laboratory analyses. Gross potassium EDTA contamination of blood samples is easily recognised by marked hyperkalaemia and hypocalcaemia. Subtle contamination is a relatively common, often unrecognised erroneous cause of spurious hyperkalaemia. In the case illustrated, it would be difficult to confidently exclude EDTA contamination based on these results alone. There is renal impairment which may explain the hyperkalaemia. The increased phosphate coupled with the renal impairment would also be an argument for the hypocalcaemia present.

In this instance, comparison with previous results was useful. The results are most likely due to renal impairment. As the patient had been admitted to the ward for a week, it was useful to be able to compare previous results. The gradual decline in renal function helped to explain the biochemical findings. As the samples were drawn on different days by different persons, the likelihood of EDTA contamination on all the days is relatively slim.

However, it is important to be cognisant that mild EDTA contamination may cause subtle shifts in results that may have negative consequences for the patient if erroneously acted on.



---

## 24. CoA trapping

|                   |                 |                  |            |
|-------------------|-----------------|------------------|------------|
| <b>Ward</b>       | Paeditric ICU   | <b>D.O.B/Age</b> | 11/03/2020 |
| <b>Consultant</b> | Prof G. vd Watt |                  |            |

Elevated propionic acid in the urine organic acid profile.

Fever with LRTI. ?COVID

Normal birth with no antenatal problems

#RVD exposed

Now:

#FTT

#LRTI. ?COVID

The patient presented with fever and LRTI which resolved after 3 -4 days of antibiotics. The patient then developed seizures with apnoeic attacks. The patient required intubation and ventilation and was transferred to ICU. The patient was noted to be having breakthrough seizures despite anticonvulsant therapy.

Further questioning revealed that the patient had become progressively drowsy with poor feeding.

Family history: No siblings noted to have had previous problem.

The patient was noted as not interacting with his environment.

CNS exam: Low GCS with upper motor neuron signs.

Other systems unremarkable.

|                      |         |        |               |
|----------------------|---------|--------|---------------|
| pH                   | 7.13 L  |        | 7.35 - 7.45   |
| pCO2                 | 2.99 L  | kPa    | 4.66 - 6.38   |
| pO2                  | 19.90 H | kPa    | 11.04 - 14.36 |
| Standard bicarbonate | 9 L     | mmol/L | 22 - 26       |
| Base excess          | -21.6 L | mmol/L | -10.0 - -2.0  |
| O2 saturation        | 100 H   | %      | 94 - 98       |
| Sodium               | 121 L   | mmol/L | 136 - 145     |
| Potassium            | 4.4     | mmol/L | 3.5 - 4.5     |
| Chloride             | 92 L    | mmol/L | 98 - 113      |
| Glucose              | 13.3    | mmol/L |               |
| Ionised calcium      | 0.80    | mmol/L |               |
| Carboxyhaemoglobin   | 3.5     | %      |               |
| Methaemoglobin       | -1.7    | %      |               |

Authorised by NL Makhalima on 28/05/2020 at 16:42

|         |        |        |         |
|---------|--------|--------|---------|
| Ammonia | 1517 H | umol/L | 40 - 80 |
|---------|--------|--------|---------|

Please note that preanalytical factors including a delay in sample reception and sample not transported on ice may cause raised ammonia results.

Trace lipaemia observed

Please repeat

|                   |                                                              |        |
|-------------------|--------------------------------------------------------------|--------|
| Total cholesterol | 1.90                                                         | mmol/L |
| Triglyceride      | 6.21                                                         | mmol/L |
| HDL cholesterol   | 0.18                                                         | mmol/L |
| LDL cholesterol   | Triglyceride level too high [>4.5mmol/l] for LDL calculation |        |

CHOLESTEROL TREATMENT TARGETS (per CV Event Risk Category):

| Risk Category:    | TC target:  | LDL-C target: |
|-------------------|-------------|---------------|
| Low/Moderate Risk | <5.0 mmol/L | <3.0 mmol/L   |
| High Risk         | <4.5 mmol/L | <2.5 mmol/L   |

Authorised by KF Sephula on 28/05/2020 at 05:29

|         |        |        |             |
|---------|--------|--------|-------------|
| Calcium | 1.24 L | mmol/L | 2.12 - 2.64 |
|---------|--------|--------|-------------|

Authorised by KF Sephula on 28/05/2020 at 05:29

|         |      |     |         |
|---------|------|-----|---------|
| Albumin | 23 L | g/L | 26 - 41 |
|---------|------|-----|---------|

Authorised by NL Makhalima on 29/05/2020 at 14:07  
Total bilirubin 5 umol/L 5 - 21

Authorised by NL Makhalima on 29/05/2020 at 14:07  
Conjugated bilirubin (DBil) 2 umol/L 0 - 5

Authorised by NL Makhalima on 29/05/2020 at 17:52  
Alanine transaminase (ALT) 106 H U/L 1 - 25

Authorised by NL Makhalima on 29/05/2020 at 14:08  
Aspartate transaminase (AST) 391 H U/L 0 - 51

Authorised by NL Makhalima on 29/05/2020 at 14:08  
Alkaline phosphatase (ALP) 382 H U/L 75 - 316

Authorised by NL Makhalima on 29/05/2020 at 14:07  
Gamma-glutamyl transferase (GGT) 44 U/L 12 - 122

Authorised by B Gool on 26/05/2020 at 16:35

CSF glucose 1.5 mmol/L

CSF glucose reference range:

CSF glucose is normally 60 - 80% of plasma glucose, in samples taken within 15 minutes of each other.

Authorised by B Gool on 26/05/2020 at 16:35

CSF protein 1.62 H g/L 0.20 - 0.80

Authorised by NL Makhalima on 26/05/2020 at 17:50

CSF adenosine deaminase 0.0 U/L

CSF ADA activity of > 6 U/L is suggestive of TB. However, other conditions such as bacterial or Cryptococcal meningitis may also produce elevated ADA levels.

**CSF Analysis:****Appearance:**

|         |              |
|---------|--------------|
| Clarity | Bloodstained |
| Clots   | Absent       |

**Cell Count:**

|              |    |     |
|--------------|----|-----|
| Polymorphs   | 0  | /uL |
| Lymphocytes  | 0  | /uL |
| Erythrocytes | 48 | /uL |

Authorised by NT Jikwana on 26/05/2020 at 14:53

**Gram Stain:**

|           |                      |
|-----------|----------------------|
| Organisms | No bacteria observed |
|-----------|----------------------|

Authorised by MG Mpotje on 28/05/2020 at 09:07

**Bacterial Culture:**

No growth after 2 days

---

Authorised by NL Makhalima on 28/05/2020 at 16:45

|                             |         |                       |               |
|-----------------------------|---------|-----------------------|---------------|
| White Cell Count            | 0.59 L  | x 10 <sup>9</sup> /L  | 5.00 - 20.00  |
| Red Cell Count              | 2.54 L  | x 10 <sup>12</sup> /L | 3.90 - 5.90   |
| Haemoglobin                 | 8.1 L   | g/dL                  | 12.0 - 21.8   |
| Haematocrit                 | 0.218 L | L/L                   | 0.340 - 0.620 |
| MCV                         | 85.7 L  | fL                    | 88.0 - 126.0  |
| MCH                         | 31.7    | pg                    | 31.0 - 37.0   |
| MCHC                        | 37.0 H  | g/dL                  | 30.0 - 36.6   |
| Red Cell Distribution Width | 14.8    | %                     |               |
| Platelet Count              | 67 L    | x 10 <sup>9</sup> /L  | 140 - 350     |
| MPV                         | 9.6     | fL                    | 7.0 - 11.4    |

|         |                                                          |
|---------|----------------------------------------------------------|
| Comment | Automated platelet count to be reviewed microscopically. |
|---------|----------------------------------------------------------|

MCHC results may be affected by lipaemia

repeated tplateet = 71

FBC comment:

No clot detected in EDTA sample

Peripheral smear to be reviewed

CT brain may be useful in assess for organic neurological



cause.

Propionic acidaemia.

DDx: Biotinidase deficiency

Propionic acidaemia is an organic acidaemia characterized by deficiency of propionyl-CoA carboxylase. Propionyl-CoA carboxylase converts propionyl-CoA to methylmalonyl-CoA. It is inherited in an autosomal recessive pattern. The metabolism of isoleucine, valine, threonine, and methionine produces propionyl-CoA. To a lesser degree, cholesterol and odd-chain fatty acids also contribute to propionyl-CoA levels. Affected individuals must follow a low-protein diet and early diagnosis improved prognosis.

The accumulation of propionyl-CoA results in significant mitochondrial CoA trapping and inhibited fatty acid oxidation. The enhanced anapleurosis of propionate and CoA trapping alters the pool sizes of tricarboxylic acid cycle (TCA) metabolites. This explains the marked hyperammonaemia that patients present with as well as potential hypoglycaemia

A high index of suspicion is required to diagnose inborn errors of metabolism (IEM). This case highlighted the importance of understanding key points in metabolic pathways. It also emphasized the correlation between catabolic stress being an initiating event in IEMs.

---

## 22. Haemolysis (intravascular)

|      |               |           |        |
|------|---------------|-----------|--------|
| Ward | Surgical ward | D.O.B/Age | 26 y.o |
|------|---------------|-----------|--------|

|                   |              |  |  |
|-------------------|--------------|--|--|
| <b>Consultant</b> | Dr H. Vreede |  |  |
|-------------------|--------------|--|--|

**Indices in serum:**

|                   |       |
|-------------------|-------|
| Haemoglobin index | 4+    |
| Bilirubin index   | Trace |
| Lipaemia index    | Trace |

Call from a clinician to assist with generating results that were being rejected due to haemolysis.

26 y.o male

#Previously well

Admitted with multiple stab wounds and had a haemo-pneumothorax on the left side. Intercostal chest drain inserted. The patient acutely decompensated after 3 days being admitted. He was noted to have metabolic acidosis, hyperlactataemia, and symptoms of shock. The patient was not on any medication besides analgesia. No previous blood transfusion. No procedure in the ward.

Patient noted to be jaundiced. Urine coke-coloured. No petechiae. Patient not bleeding from any wound sites.

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|        |       |        |           |
|--------|-------|--------|-----------|
| Sodium | 127 L | mmol/L | 136 - 145 |
|--------|-------|--------|-----------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|           |       |        |           |
|-----------|-------|--------|-----------|
| Potassium | 6.5 H | mmol/L | 3.5 - 5.1 |
|-----------|-------|--------|-----------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|      |        |        |           |
|------|--------|--------|-----------|
| Urea | 20.0 H | mmol/L | 2.1 - 7.1 |
|------|--------|--------|-----------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|                     |       |                            |          |
|---------------------|-------|----------------------------|----------|
| Creatinine          | 272 H | umol/L                     | 64 - 104 |
| eGFR (MDRD formula) | 25    | mL/min/1.73 m <sup>2</sup> |          |

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|         |      |        |             |
|---------|------|--------|-------------|
| Calcium | 2.15 | mmol/L | 2.15 - 2.50 |
|---------|------|--------|-------------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|           |      |        |             |
|-----------|------|--------|-------------|
| Magnesium | 0.87 | mmol/L | 0.63 - 1.05 |
|-----------|------|--------|-------------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|                     |        |        |             |
|---------------------|--------|--------|-------------|
| Inorganic phosphate | 2.05 H | mmol/L | 0.78 - 1.42 |
|---------------------|--------|--------|-------------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|         |      |     |         |
|---------|------|-----|---------|
| Albumin | 24 L | g/L | 35 - 52 |
|---------|------|-----|---------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|                 |      |        |        |
|-----------------|------|--------|--------|
| Total bilirubin | 44 H | umol/L | 5 - 21 |
|-----------------|------|--------|--------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|                             |   |        |       |
|-----------------------------|---|--------|-------|
| Conjugated bilirubin (DBil) | 3 | umol/L | 0 - 3 |
|-----------------------------|---|--------|-------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|                            |       |     |         |
|----------------------------|-------|-----|---------|
| Alanine transaminase (ALT) | 342 H | U/L | 10 - 40 |
|----------------------------|-------|-----|---------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|                            |    |     |          |
|----------------------------|----|-----|----------|
| Alkaline phosphatase (ALP) | 88 | U/L | 53 - 128 |
|----------------------------|----|-----|----------|

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

|             |      |     |             |
|-------------|------|-----|-------------|
| Haptoglobin | 0.46 | g/L | 0.30 - 2.00 |
|-------------|------|-----|-------------|

Authorised by Instrument on 06/11/2019 at 04:38

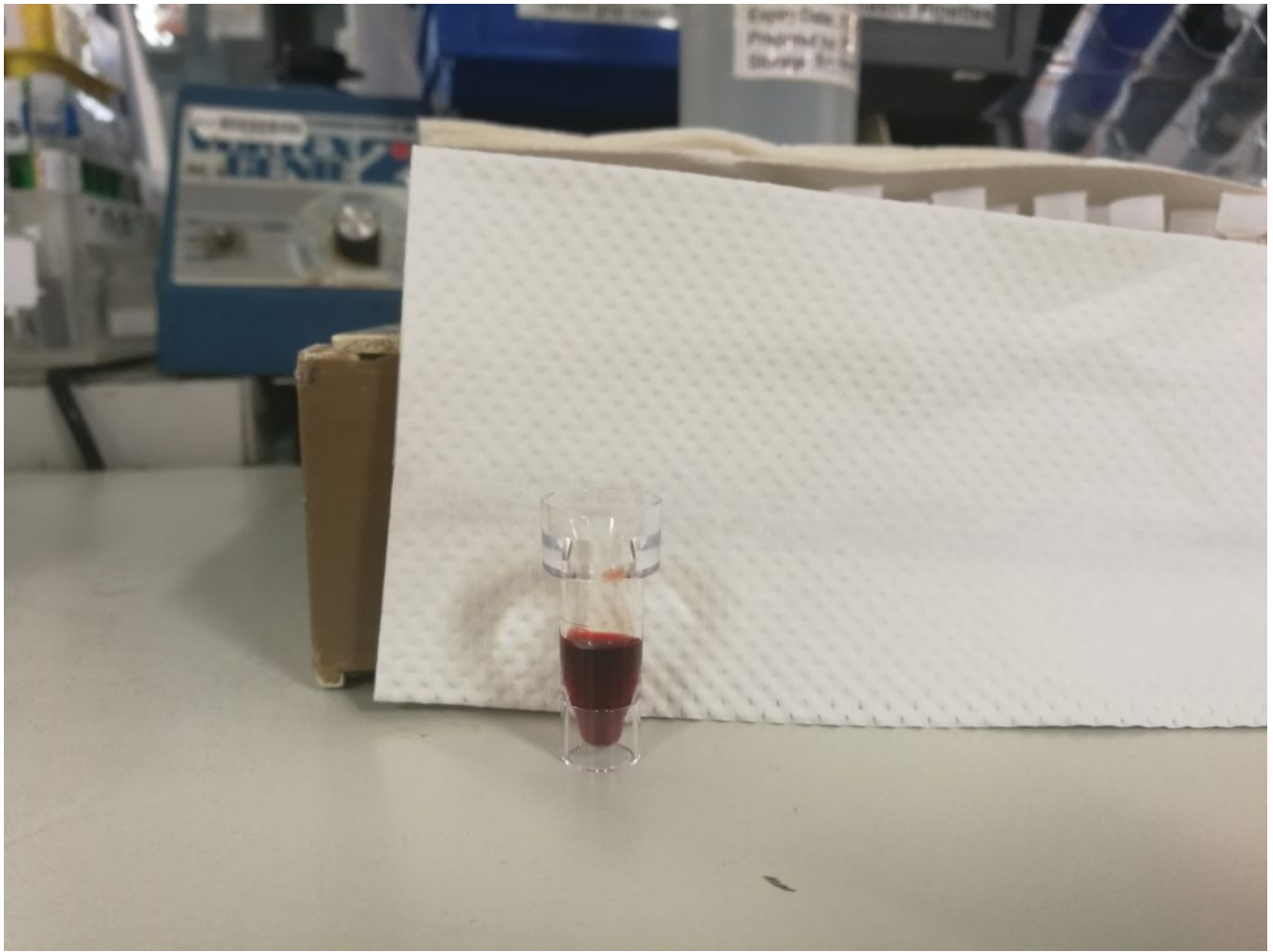
Indices in serum:

|                   |       |
|-------------------|-------|
| Haemoglobin index | 4+    |
| Bilirubin index   | Trace |
| Lipaemia index    | Trace |

Authorised by Dr TA Gcingca on 06/11/2019 at 14:21

Chemistry comment:

Gross haemolysis is present. The decreased haptoglobin level points toward intravascular haemolysis but please treat results with reserve and correlate with clinical findings.



Patient sample after centrifugation





Gross haemolysis

CXR: haemopneumothorax on the left.

?sepsis ?toxin introduced through stab wounds

Intravascular haemolysis results in the release of cell-free haemoglobin, red blood cell (RBC) stroma, and non-stroma proteins. Free haemoglobin binds nitric oxide (NO) at rate 1000 times that of RBC. Haemoglobin scavenging leads to decreased bioavailability of NO and thus vasoconstriction and alterations in capillary response to hypoxia. RBC stroma, which is the cytoskeletal framework supporting haemoglobin, can also contribute to DIC pathogenesis via activation of platelets and coagulation cascade. RBC stroma has also been shown to increase blood pressure and is toxic to the glomerulus and renal tubule and thus can cause acute renal failure. Ultimately, increased cytokines and hypotension stimulate a compensatory sympathetic nervous system response contributing to renal, splanchnic, and cutaneous

vasoconstriction that, in combination with pathophysiology described above, leads to shock and circulatory collapse.

Marked increase of lactate dehydrogenase and haemosiderinuria are typical of intravascular haemolysis. Several haemolytic markers are available to guide the differential diagnosis and to monitor treatment of haemolytic conditions. They include increased reticulocytes (an indicator of marrow compensatory response) elevated lactate dehydrogenase, reduced haptoglobin, and unconjugated hyperbilirubinemia.

However, increased reticulocytes, lactate dehydrogenase, and bilirubin, as well as reduced haptoglobin, are observed in conditions other than haemolysis that may confound the clinical picture.

Haptoglobin is a positive acute-phase reactant. It is a protein that binds irreversibly to free (oxy)haemoglobin liberated into the plasma during intravascular haemolysis. The haptoglobin-haemoglobin complex is removed rapidly by the reticuloendothelial system to prevent loss of haemoglobin in urine. Low levels are a diagnostic indicator of intravascular haemolysis (but may be low in liver disease or with endogenous or exogenous oestrogen). Elevated levels are associated with acute phase response, nephrotic syndrome and with corticosteroids.

It is interesting to note in this patient that his result is in the lower level of normal, pointing towards the possibility that haptoglobin may be markedly decreased

---

## 4. PSA

|            |                     |           |            |
|------------|---------------------|-----------|------------|
| Ward       | Casualty Department | D.O.B/Age | 04/12/1940 |
| Consultant |                     |           |            |

Prostate-specific Ag (PSA) 949.50 H  
ug/L <4.00

Urinary retention.

Request form: Lower urinary tract symptoms and urinary retention.

Important clinical findings to assess for include:

**General:** Temporal wasting, signs of urinary incontinence (e.g. any leaking noted, need to wear sanitary products)

**Abdominal:** Assess for masses, palpable bladder from retention

**P.R:** Assess prostate for size, consistency, tenderness.

**CNS:** Assess for any neurological fallout as prostatic metastasis tend to metastasize to the lower vertebrae.

Creatinine 83  
umol/L 64 – 104  
eGFR (MDRD formula) >60  
mL/min/1.73 m2

White Cell Count 5.01 x  
109/L 3.92 – 10.40

|                             |               |             |          |
|-----------------------------|---------------|-------------|----------|
| Red Cell Count              |               | 5.39        | x        |
| 10 <sup>12</sup> /L         | 4.50 – 5.50   |             |          |
| Haemoglobin                 |               | 15.5        |          |
| g/dL                        | 13.0 – 17.0   |             |          |
| Haematocrit                 |               | 0.485       |          |
| L/L                         | 0.400 – 0.500 |             |          |
| MCV                         |               | 90.0        |          |
| fL                          | 83.1 – 101.6  |             |          |
| MCH                         |               | 28.8        |          |
| pg                          | 27.8 – 34.8   |             |          |
| MCHC                        |               | <b>32.0</b> | <b>L</b> |
| g/dL                        | 33.0 – 35.0   |             |          |
| Red Cell Distribution Width |               | 13.2        |          |
| %                           | 12.1 – 16.3   |             |          |
| Platelet Count              |               | 226         | x        |
| 10 <sup>9</sup> /L          | 171 – 388     |             |          |

#### PATHOLOGICAL DIAGNOSIS:

Prostate, biopsy:

Adenocarcinoma.

Imaging studies may be necessary if there is a concern for metastasis and these will be guided by the clinical presentation e.g. CXR if metastasis to the lungs is suspected vs MRI if there is a concern of vertebral collapse.

Prostatic adenocarcinoma.

- Prostate-specific antigen (PSA) is a protein produced by normal prostatic cells. The majority of PSA is produced by the glands in the transitional zone of the prostate (BPH). The peripheral zone, where 80% of prostate cancers originate, produces very little PSA.
- An enlarged prostate can cause obstructive uropathy. The creatinine values in this patient do not suggest renal impairment though a baseline creatinine would be required to assess this.
- PSA is used for screening, diagnosis as well as

monitoring of prostate related disease processes. PSA is an organ-specific, not a cancer-specific marker. It is useful in detection, staging and monitoring of prostate cancer.

- To improve diagnostic accuracy when PSA is between 4-10ug/L ("grey zone"), free PSA is measured and the free/total PSA ratio is calculated. Most normal PSA is protein-bound, and in prostatic cancer, a greater proportion is unbound. A free/total PSA ratio <0.25 increases the likelihood of cancer.

---

## 21. APT

|                   |                |                  |        |
|-------------------|----------------|------------------|--------|
| <b>Ward</b>       | Maternity Ward | <b>D.O.B/Age</b> | 27 y.o |
| <b>Consultant</b> | Dr C. Hudson   |                  |        |

APT test positive.

?haemolytic disease of the newborn

27 y.o. female

G2P1 at 34 weeks

RH negative with Rhesus iso-immunization. Anti-D titres 1:128. (Blood group AB negative). Coombs test positive. Risk of haemolytic disease of the new-born.

Not applicable/difficult to examine foetus in-utero. Ultrasound of the middle cerebral artery peak systolic velocity was suggestive that the baby was anaemic.

U/s guided chordocentesis done and foetal blood sample obtained in utero. FBC whilst in utero showed Hb: 9.8.

Clinician requested APT test to ensure that foetal sample

obtained during chordocentesis.

Haematology did Kleihauer Betke Test and it showed 100% foetal haemoglobin. APT test also correlated and showed foetal haemoglobin.

Ultrasound: middle cerebral artery peak systolic velocity suggestive that the baby was anaemic. U/s guided chordocentesis done and foetal blood sample obtained in utero. FBC whilst in utero showed Hb: 9.8.

APT test confirmed that foetal blood had been obtained during chordocentesis. It also correlated with the Kleihauer Betke done by haematology.

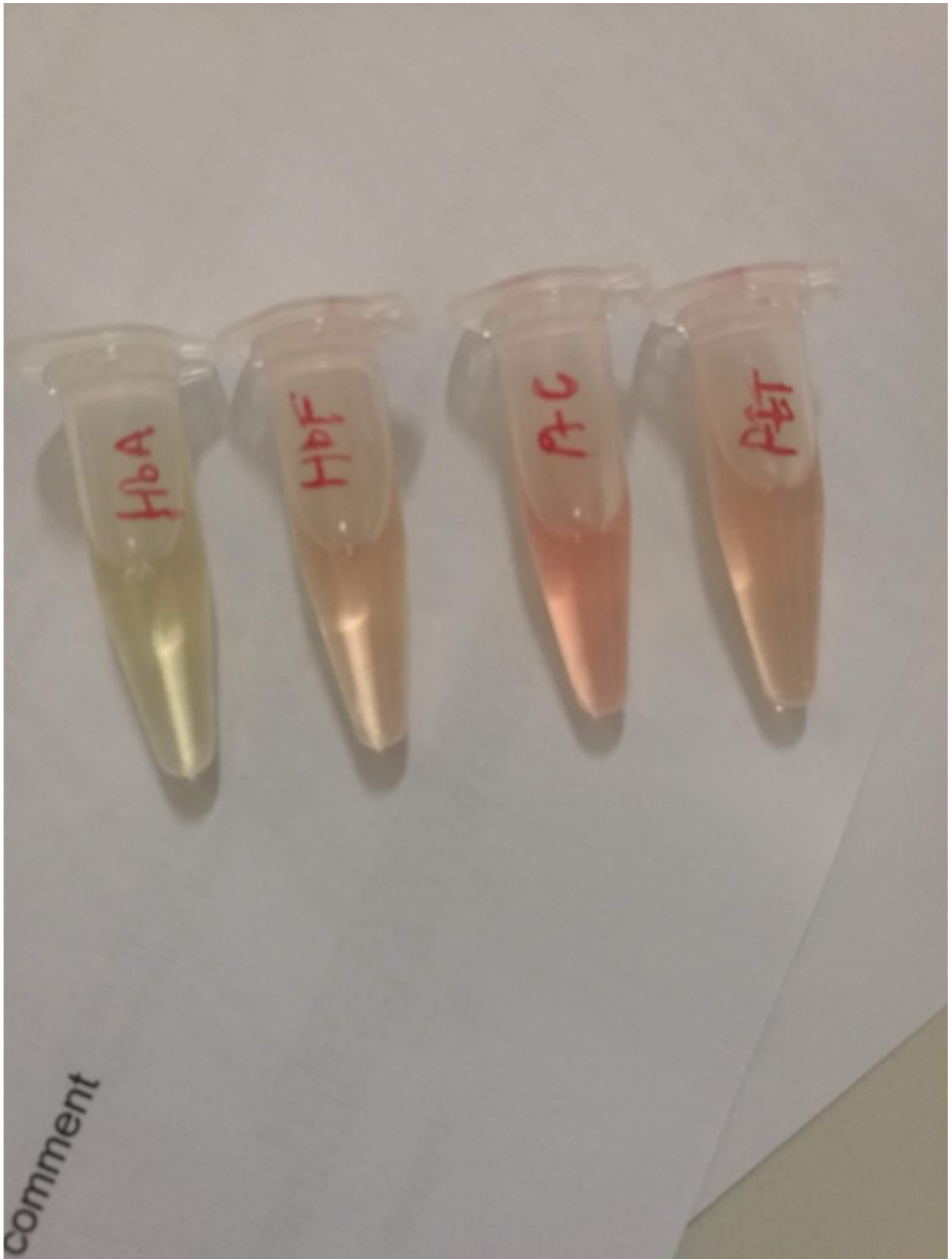


Image 1

HbA: adult haemoglobin; HbF: foetal haemoglobin; PtC: patient control; PtT: test control.

Note the slight green tinge of HbA

Principles of the APT test: Sodium hydroxide (NaOH) denatures adult oxyhaemoglobin to haematin (with a colour change from pink to yellow green). Foetal haemoglobin resists alkaline denaturation by NaOH and maintains a pink colour. If adult haemoglobin (HbA) is present in the sample, it turns yellow and then green within two minutes of the addition of sodium hydroxide. Any pink colour that persists for longer than 2 minutes indicates foetal haemoglobin (HbF) is present in the sample.

The Kleihauer Betke Test is an acid-elution assay performed on maternal blood to determine the amount of HbF that has passed into maternal circulation. The process exposes maternal blood smear to an acid solution. HbF, being resistant to the acid, remains intact, whereas HbA is removed. Following this, the smear is stained via Shepard's method. The foetal red blood cells are left rose-pink in colour, and the maternal cells appear "ghost-like" due to the absence of staining. This test is done by Haematology. Other ways of differentiating maternal from foetal blood is examining the MCV. The foetus has a larger MCV. This is nonspecific though as the mother may have macrocytic anaemia.

---

## 3. Hyperammonaemia

|                   |              |                  |            |
|-------------------|--------------|------------------|------------|
| <b>Ward</b>       | Medical ward | <b>D.O.B/Age</b> | 16/07/1950 |
| <b>Consultant</b> |              |                  |            |

Ammonia

251 H

umol/L

[11 – 35]

|                                                                                               |
|-----------------------------------------------------------------------------------------------|
| Specimen request form has hepatic encephalopathy written as the diagnosis/reason for request. |
|-----------------------------------------------------------------------------------------------|



Unable to obtain.

Unable to obtain.

|                                  |               |                |             |
|----------------------------------|---------------|----------------|-------------|
| Sodium                           | 143           | mmol/L         | [136 – 145] |
| Potassium                        | 4.4           | mmol/L         | [3.5 – 5.1] |
| Urea                             | <b>28.1 H</b> | mmol/L         | [2.1 – 7.1] |
| Creatinine                       | <b>359 H</b>  | umol/L         | [64 – 104]  |
| eGFR (MDRD formula)              | 15            | mL/min/1.73 m2 |             |
| INR                              |               | 2.77           |             |
| Total bilirubin                  | <b>54 H</b>   | umol/L         | [5 – 21]    |
| Conjugated bilirubin (DBil)      |               | <b>36 H</b>    | umol/L      |
|                                  |               | [0 – 3]        |             |
| Alanine transaminase (ALT)       |               | 34             | U/L         |
|                                  |               | [10 – 40]      |             |
| Aspartate transaminase (AST)     |               | <b>113 H</b>   | U/L [15 –   |
|                                  |               | 40]            |             |
| Alkaline phosphatase (ALP)       |               | 105            | U/L         |
|                                  |               | [53 – 128]     |             |
| Gamma-glutamyl transferase (GGT) |               | 33             |             |
|                                  | U/L           | <68            |             |

Unremarkable CMP. Elevated WCC of 11 otherwise normal FBC.  
Further investigations not requested on Trakcare. ?patient  
discharge vs transfer vs demise

?Fulminant liver cirrhosis

?End stage liver disease

Most ammonia dealt with by the liver is produced by gut organisms. Protein degradation forms a smaller contribution. Ammonia in high concentrations is neurotoxic. It is detoxified by the liver to urea via means of the urea cycle, and urea is subsequently excreted in the urine. Pre-analytical factors including a delay in sample reception and sample not transported on ice may cause raised ammonia results. Other pre-analytical factors to consider include:

- No smoking by the person collecting the sample or the

patient the sample is being collected from.

- Tourniquet should not be applied tightly or for too long (no tourniquet application ideal).
- Collected in an EDTA container.
- Must reach the lab within 15 to 20 minutes of being collected on ice.
- Patient should be fasted.

This patient has mildly deranged liver function tests and a prolonged INR suggesting liver disease which may be contributing to the hyperammonaemia. The unremarkable elevation in the liver enzymes may be due to a decrease of viable hepatocytes.

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## 20. Alpha-fetoprotein

|            |                |           |            |
|------------|----------------|-----------|------------|
| Ward       | Emergency unit | D.O.B/Age | 07/08/1968 |
| Consultant | Dr C. Hudson   |           |            |

Request form: No clinical information provided

Unavailable.

Unavailable.

|                     |           |     |            |             |
|---------------------|-----------|-----|------------|-------------|
| Sodium              |           |     | <b>130</b> | <b>L</b>    |
| mmol/L              | 136 – 145 |     |            |             |
| Potassium           |           |     | 3.7        |             |
| mmol/L              | 3.5 – 5.1 |     |            |             |
| Urea                |           |     | 2.9        |             |
| mmol/L              | 2.1 – 7.1 |     |            |             |
| Creatinine          |           |     | 64         |             |
| umol/L              | 49 – 90   |     |            |             |
| eGFR (MDRD formula) |           | >60 |            | mL/min/1.73 |

m2

**Glycated haemoglobin (HbA1c):**

|                                  |             |        |
|----------------------------------|-------------|--------|
| Glycated haemoglobin (NGSP)      | 6.5         | %      |
| Glycated haemoglobin (IFCC)      | 48          |        |
| mmol/mol                         |             |        |
| Estimated average glucose (eAG)  | 7.8         | mmol/L |
| Calcium                          | 2.20        |        |
| mmol/L                           | 2.15 – 2.50 |        |
| Total protein                    | 86          | H      |
| g/L                              | 60 – 78     |        |
| Albumin                          | 28          | L      |
| g/L                              | 35 – 52     |        |
| Total bilirubin                  | 26          | H      |
| umol/L                           | 5 – 21      |        |
| Conjugated bilirubin (DBil)      | 25          | H      |
| umol/L                           | 0 – 3       |        |
| Alanine transaminase (ALT)       | 65          | H      |
| U/L                              | 7 – 35      |        |
| Aspartate transaminase (AST)     | 444         | H      |
| U/L                              | 13 – 35     |        |
| Alkaline phosphatase (ALP)       | 568         | H      |
| U/L                              | 42 – 98     |        |
| Gamma-glutamyl transferase (GGT) | 662         | H      |
| U/L                              | <40         |        |
| Lipase                           | 91          | H      |
| U/L                              | 13 – 60     |        |
| Alpha-feto protein (AFP)         | 545010.0    | H      |
| ug/L                             | 0.0 – 7.0   |        |
| Thyroid stimulating hormone      | 6.61        | H      |
| mIU/L                            | 0.27 – 4.20 |        |
| Thyroxine (free T4)              | 13.7        |        |
| pmol/L                           | 12.0 – 22.0 |        |

|                             |               |         |   |
|-----------------------------|---------------|---------|---|
| White Cell Count            |               | 8.93    | x |
| 109/L                       | 3.90 – 12.60  |         |   |
| Red Cell Count              |               | 2.92 L  | x |
| 1012/L                      | 3.80 – 4.80   |         |   |
| Haemoglobin                 |               | 9.4 L   |   |
| g/dL                        | 12.0 – 15.0   |         |   |
| Haematocrit                 |               | 0.278 L |   |
| L/L                         | 0.360 – 0.460 |         |   |
| MCV                         |               | 95.2    |   |
| fL                          | 78.9 – 98.5   |         |   |
| MCH                         |               | 32.2    |   |
| pg                          | 26.1 – 33.5   |         |   |
| MCHC                        |               | 33.8    |   |
| g/dL                        | 32.7 – 34.9   |         |   |
| Red Cell Distribution Width |               | 19.5 H  |   |
| %                           | 12.4 – 17.3   |         |   |
| Platelet Count              |               | 246     | x |
| 109/L                       | 186 – 454     |         |   |

Medical Validation : (Authorise By Episode)

Option Mode Inquire Function Audit Print Help Result

Episode No. [REDACTED] MRN [REDACTED] HPRN [REDACTED] Stat [REDACTED]

F 51 y 07/08/1968

Hos Groote Schuur Hospital wc GSH 021 404 9111 Collection 30/12/2019 13:04

Wrd C15 Emergency Unit 404 5208 / 09 Received 30/12/2019 13:18

Doc [REDACTED] Registered 30/12/2019 13:20 ePR Detail

Visit Test Set(s)

- [A] TREQ <M entry>
- [A] SPARE1 <A entry>
- [A] HBA1C <A entry>
- [A] CA <A entry>
- [A] LIPASE <A entry>
- [E] AFP # <M entry> (VQ:CR)
- [A] TSH <A entry>

(\* Curr.) (# In List)

Update Amend Clear Notes Graph

Authorise Fully Authorise Cancel

| Test Set | Staff Notes                         | Test Item                | Result    | Units  | Normal Values | Previous Result 1 | Previous Result 2 | Previous Result 3 | Previous Result 4 | Previous Result 5 |
|----------|-------------------------------------|--------------------------|-----------|--------|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| ALB      |                                     | Albumin                  | 28        | g/L    | 35 - 52       |                   |                   |                   |                   |                   |
| TBIL     |                                     | Total bilirubin          | 26        | umol/L | 5 - 21        |                   |                   |                   |                   |                   |
|          |                                     | Total bilirubin auto com |           |        |               |                   |                   |                   |                   |                   |
| CBIL     |                                     | Conjugated bilirubin (Df | 25        | umol/L | 0 - 3         |                   |                   |                   |                   |                   |
| ALT      |                                     | Alanine transaminase (A  | 65        | U/L    | 7 - 35        |                   |                   |                   |                   |                   |
| AST      |                                     | Aspartate transaminase   | 444       | U/L    | 13 - 35       |                   |                   |                   |                   |                   |
| ALP      |                                     | Alkaline phosphatase (A  | 568       | U/L    | 42 - 98       |                   |                   |                   |                   |                   |
| GGT      |                                     | Gamma-glutamyl transfe   | 662       | U/L    | <40           |                   |                   |                   |                   |                   |
| LIPASE   |                                     | Lipase                   | 91        | U/L    | 13 - 60       |                   |                   |                   |                   |                   |
| AFP      | <input checked="" type="checkbox"/> | Alpha-feto protein (AFP  | 545,010.0 | ug/L   | 0.0 - 7.0     |                   |                   |                   |                   |                   |
|          |                                     | Machine (Serum)          | COB       |        |               |                   |                   |                   |                   |                   |
|          |                                     | AFP auto comment         | AFPCOB    |        |               |                   |                   |                   |                   |                   |
| TSH      |                                     | Thyroid stimulating hor  | 6.61      | mIU/L  | 0.27 - 4.20   |                   |                   |                   |                   |                   |
| FT4      |                                     | Thyroxine (free T4)      | 13.7      | pmol/L | 12.0 - 22.0   |                   |                   |                   |                   |                   |
| SIND     |                                     | Serum haemoglobin inc    | 0         |        |               |                   |                   |                   |                   |                   |
|          |                                     | Serum bilirubin index    | 1         |        |               |                   |                   |                   |                   |                   |
|          |                                     | Serum lipaemia index     | 0         |        |               |                   |                   |                   |                   |                   |
|          |                                     | Serum haemoglobin va     | 0.00      |        |               |                   |                   |                   |                   |                   |

Staff Notes : C136

Help

[30/12/2019 22:34] [REDACTED] AFP Checked.

R1 - >1210  
R2 - >60500 1:50  
R3 - >121000 1:100  
R4 - >484000 1:400  
R5 - 545010 1:1000  
[30/12/2019 22:42 bilqeess.jacobs] Result verified

OK Cancel

Abdominal ultrasound +/- CT scan may be helpful in detecting presence of liver mass +/- intra-abdominal masses.

## Final diagnosis

?Hepatocellular carcinoma

This case allowed me to become familiar with the concepts related to limitations of an assay. Having come across the need for dilution and the concept of high-dose hook effect, I found it interesting to see the gradual increase in AFP value as further dilutions were done. These are terms and concepts that this case allowed me to become familiar with.

**Limit of Blank:** This is the highest apparent analyte concentration expected to be found when replicates of a blank sample (containing no analyte) are tested. Detects “noise” that could interfere with the result.

**Limit of Detection:** This refers to the lowest analyte concentration likely to be reliably distinguished from the limit of blank and at which detection is feasible. LoD is determined using measured limit of blank, and test replicates known to contain a low concentration of an analyte.

**Limit of Quantitation:** This is the lowest concentration at which the analyte can not only be reliably detected but also at which some predefined goals for precision and bias are met. The LoQ may be equivalent to the LoD or it could be at a higher concentration. This is the limit that is clinically significant.