Drip line contamination – Ringers Lactate

A case of drip line contamination.

Albumin Assay – Bromocresol Green method

Practical assay for albumin measurement

Total Protein assay – Bradford

A practical experiment to illustrate the measurement of total protein in serum using the Bradford assay

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Hypernatremia

Case of a child with extreme hypernatremia.

Prolactin

HOSP #		WARD	ENT Clinic
CONSULTANT		DOB/AGE	35 Y Male

Abnormal Result

Prolactin 10 986.0 ug/L (4-15.2)

Dilutions:

1/10 >4700;

1/100 = 10821;

1/50 = 10 986.

Presenting Complaint

Epistaxis

History

Patient with epistaxis referred to the ENT specialist clinic. No relevant medication history.

Examination

35 y male with a large left post-nasal space mass, a vascular mass involving the pituitary fossa.

?NBL (non-benign lesion)

?Sinonasal malignancy

?Pituitary Tumour

Laboratory Investigations

TSH 0.91 pmol/L (0.27-4.20)

Free T4 15.7 pmol/L (12-22)

FSH 0.8 IU/L ↓ (1.5-12.4)

LH 0.2 IU/L ↓ (1.7-8.6)

Testosterone 0.2 nmol/L ↓ (8.6-29.0)

PTH 1.7 pmol/L (1.6-6.9)

Prolactin measuring method:

The Elecsys prolactin sandwich immunoassay uses two monoclonal antibodies directed against human prolactin.

R1 = biotinylated antibody – recognizes the N-terminal end of the
prolactin molecule

R2 – ruthenium complexed antibody probably reacts with a region in the
middle of the prolactin molecule.

1st incubation: a biotinylated monoclonal prolactin-specific antibody and a monoclonal prolactin-specific antibody labeled with a ruthenium
complex form a sandwich complex.

2nd incubation: after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of
biotin and streptavidin.

Reaction mixture aspirated into the measuring cell where

microparticles
are magnetically captured into the surface of the electrode. Unbound substances are then removed with
ProCell.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier, results
calculated by a standard curve.

Other Investigations

Monomeric prolactin – 7744 ug/L (70% recovery after PEG precipitation)

Biopsy: confirmed tumour stained strongly positive
with prolactin suggesting a prolactinoma.

Final Diagnosis

Pituitary Macroprolactinoma

Take Home Messages

Sandwich immunoassays are prone to high dose hook-effect. There are
various ways to overcome this effect. (This will later be expanded on – see AFP
/ Beta-HCG).

Prolactin appears in the serum as:

1. Active monomeric
   prolactin (“little”) (80%) 23kDa
2. Inactive dimeric
   prolactin (“big”) (5-20%) 50-60kDa
3. Low activity
   tetrameric prolactin (“big-big”) (0.5-5%) 150-170kDa

Precipitation by PEG yields the active monomeric prolactin, expressed as a percentage recovery after precipitation. Big-big prolactin consists of an antigen-antibody complex of monomeric prolactin-immunoglobulin G and is defined as macroprolactin. This has a long half-life in blood when compared to normal prolactin and gives false high readings of prolactin, leading to unnecessary investigations in certain cases. A high prolactin should thus be confirmed by doing a PEG precipitation.

Fluid Triglycerides

A case of high fluid triglycerides

ACTH

HOSP #		**WARD**	G16 Medical Ward
CONSULTANT		**DOB/AGE**	54 y Female

Abnormal Result

21/08/2018 Two ACTH tests (referred to another laboratory) and two Cortisol levels (at our laboratory) were done.

At first it was thought to be a dexamethasone suppression test, but then realized the clinicians were suspecting hypopituitarism.

10h05: **ACTH 0.7 pmol/L ↓** (1.6-13.9) Cortisol 8 nmol/L ↓ (Morning: 133- 537; Afternoon 68 – 327)

10h35: ACTH 1.8 pmol/L N (1.6-13.9)
Cortisol 68 nmol/L ↓ (Morning: 133- 537; Afternoon 68 – 327)

Presenting Complaint

? hypopituitarism

History

Known with a pituitary macroadenoma, previously seen at the Radiotherapy clinic in 2016.

Examination

No clinical info available.

For Primary adrenal insufficiency one would expect: Hyperpigmentation (due to ↑ ACTH), +/- hyperkalemia/hyponatremia (aldosterone effect), +/- virilization.

For Secondary adrenal insufficiency there is subtle symptoms, electrolytes are not deranged significantly because aldosterone function is preserved. See table on Bishop 7th ed. p. 459.

Laboratory Investigations

Measurement of

plasma ACTH concentration is used to assess Cushing's disease, adrenal tumors, ectopic ACTH-producing tumors, Addison's disease, Nelson's syndrome, and hypopituitarism.

The laboratory diagnosis of hypopituitarism, however is relatively straightforward. In contrast to the primary failure of an endocrine gland that is accompanied by dramatic increases in circulating levels of the corresponding pituitary tropic hormone, secondary failure (hypopituitarism) is associated with low or normal levels of tropic hormone. This is the diagnosis in this case with the history of previous radiotherapy which was given for a macro-adenoma.

Other Investigations

Free T4 on 19/04/2018 was 7.8 pmol/L (12-22), also suggesting possible hypopituitarism, although a TSH would be helpful.

Final Diagnosis

Hypopituitarism confirmed.

Take Home Messages

Dexamethasone suppression test need only measurement of cortisol, not accompanying ACTH, except in extended work-up however, where a Cosyntropin (CRH) stimulation test can be done to distinguish between pituitary or hypothalamic insufficiency.

Evaluation of pituitary function need the Primary hormone (Cortisol) as well as the tropic hormones from the pituitary (ACTH).

Paracetamol Overdose

HOSP #		**WARD**	C15 Casualties
Consultant		**DOB/AGE**	33 year Female

Abnormal result

Paracetamol 25ug/ml (163 umol/L) Serum osmolarity 310mmol/L

Presenting Complaints

Brought to casualties with stupor from Mitchells Plein Hospital.

History

33 y female presented with stupor after ingestion of an unknown amount of pills. Empty container of Amitriptiline and Paracetamol was found with her.

Examination

Non-specific neurologic signs, but delirium present. Patient did have an episode of vomiting. No pathological signs on abdominal examination.

Laboratory Investigations

12/08/2018: Na 156 mmol/L (H) Urea 4.2mmol/L Tot. Bili 4 umol/L K 1.9 mmol/L (L) Creat 88 umol/L ALT 82 U/L Cl 97.9 mmol/L (L) Gluc 3.52 mmol/L AST 238 U/L Ammonia 35 umol/L Bicarb 16.6 mmol/L (L) Osmol 310 mmol/L (H) Osmolar gap: -10 mM Anion Gap: 47 mmol/L

Marked elevation of hepatocellular enzymes, ductal enzymes within normal range. Within the course of three days the patient developed Klebsiella Pneumoniae on intubation in ICU with DIC and marked renal failure (Creat 506, Urea 26.8) and demised in ICU 3 days after admission, although liver enzymes were not markedly more deranged as initial presentation.

Paracetamol: The Paracetamol level was never repeated after admission. Doing an in-house experiment with calibrator and spiking the calibrator samples with N-acetylcysteine correlating with therapeutic plasma levels, I demonstrated that our method on the Roche analyzer, with the enzymatic assay, causes a clinically significant negative interference in the measured paracetamol.

The enzymatic assay principle:

arylacylamidase hydrolysis o-cresal + periodate catalyst

Acetaminophen→ p-aminophenol+acetate → indophenol (measured @600nm)

Other Investigations

Tricyclic antidepressant levels 58 ug/L ([TCA] in overdose patients range from 29-1732ug/L, but has not been found to

correlate to clinical outcome, unless plasma level is more than 1000ug/L).

Final Diagnosis

Klebsiella Sepsis (confirmed on blood culture 1 day after death) DIC with marked renal failure.

Take Home Messages

- Paracetamol reporting units must be confirmed, we generally use ug/ml, but it has created confusion previously, as nomograms used in South Africa generally use ug/ml.
- N-acetyl cysteine may cause negative interference with the measurement of paracetamol in the enzymatic assay. Sampling for Paracetamol levels should thus be done before an IV dose of NAC is given to eliminate this possible error. National guidelines with toxicology will likely be amended.