THAMES AUDIT GROUP

Audit of Macroanalytes

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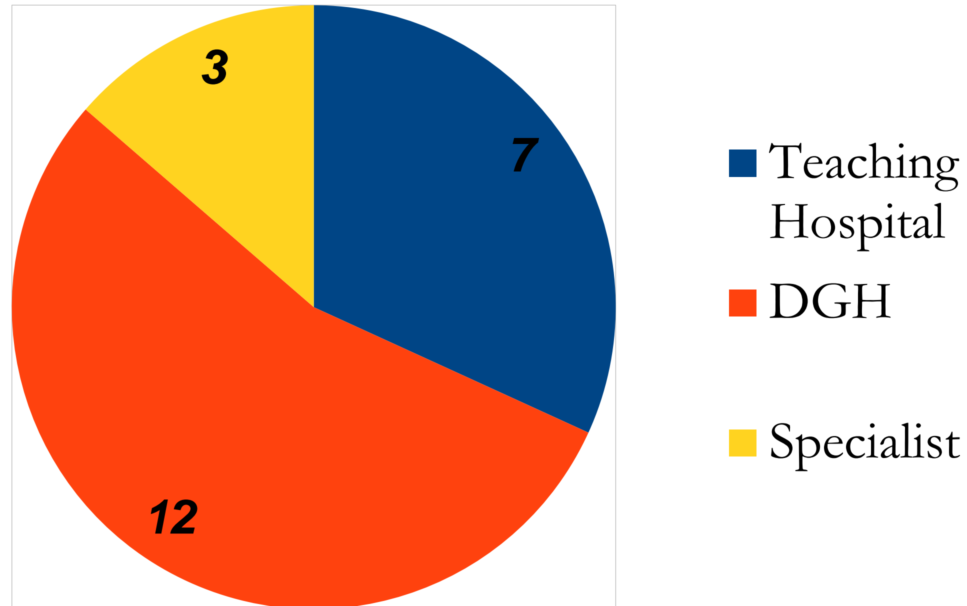
Previous Cortisol Audit 2018
Participants n=22

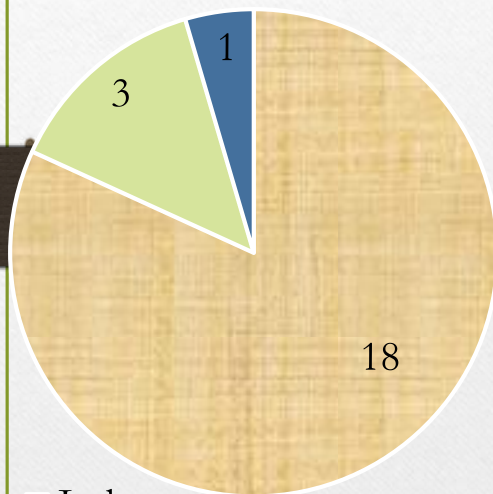


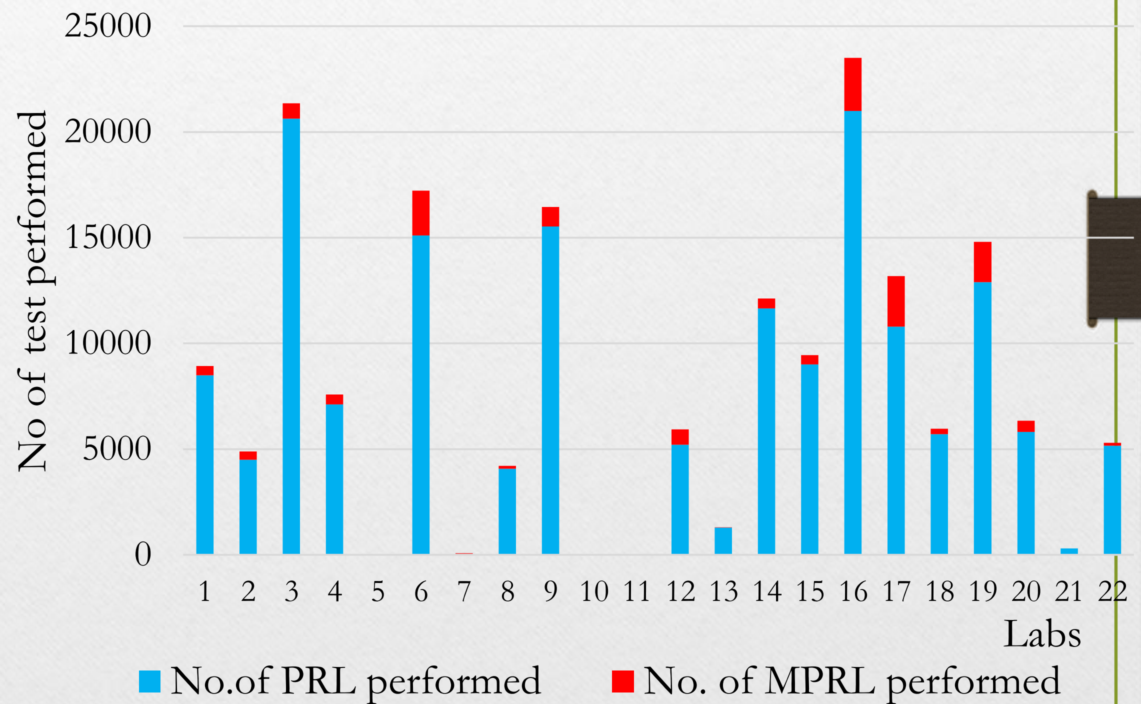
13 lab participated in
2 lab implemented changes

Part 3. Macroprolactin - MPRL

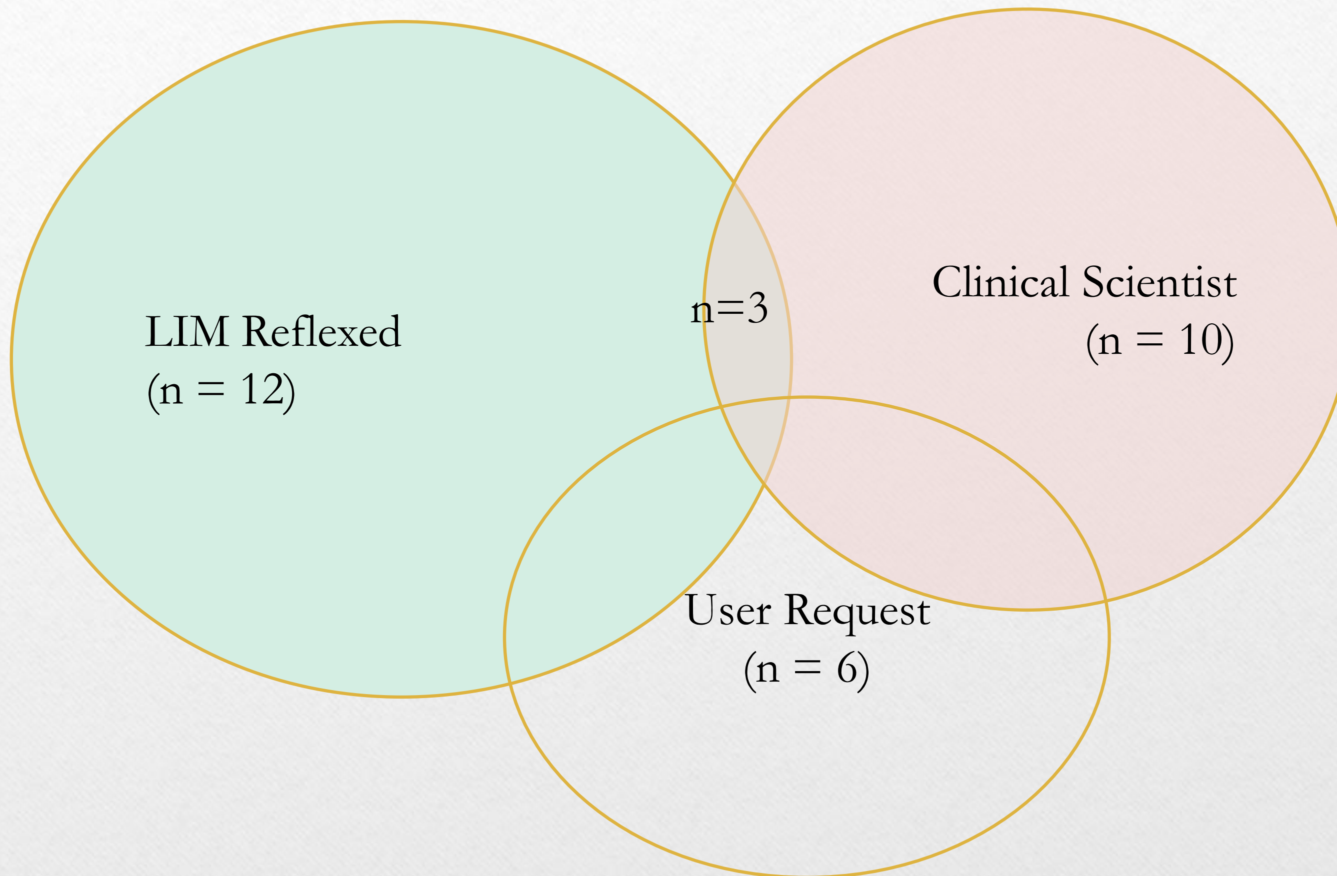
Section 1 - **Requesting**

Q-A1&2: Estimated workload each year





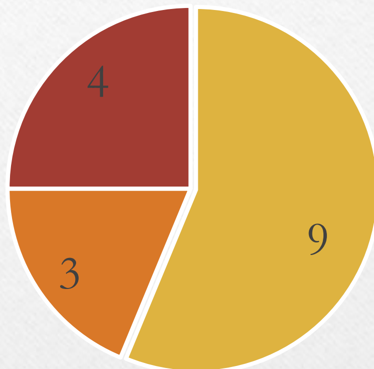
Q-A3: How are MPRL screens requested?



Q-A4: Criteria for requesting/reflexing a MPRL

1) PRL results

PRL Cut-off for reflexing MPRL

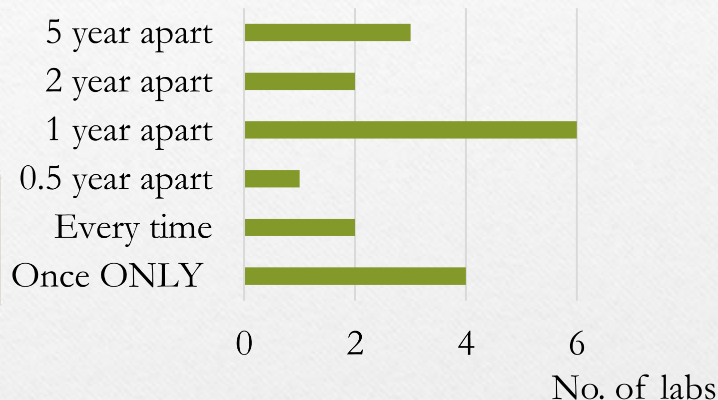


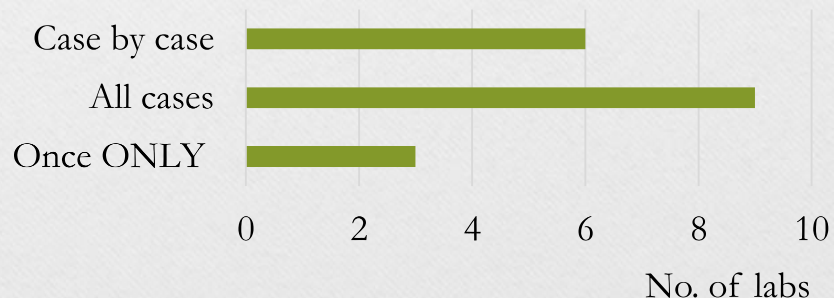
700 F&M
1000 F&M
Based on PRL RR

Recommend:
All laboratories should screen hyperprolactinaemic samples for the presence of macroprolactin, using >1000mU/L(females) and >700 mU/L(males)as a cut-off. Rarely it may be necessary to consider screening in patients with confirmed and persistent prolactin concentrations between the reference range and the suggested cut-offs in whom other causes have been excluded.

Q-A5-6: Criteria for requesting/reflexing a MPRL

2) Repeat Intervals





Recommend:

- Consider extending the repeat testing intervals for previous Neg MPRL to reduce workload.
- Positive MPRL should all be repeated so that BMP could be monitored

Q-A7: Case Study

Date of Sample	Now	10.2019	02.2019	10.2018	02.2018
Prolactin mU/L	1900	450	400	1700	1900
MPRL Recovery					70%

Question to discuss:

Would it possible to develop positive MPRL after prolactin normalised ?

Part 3. Macroprolactin - MPRL

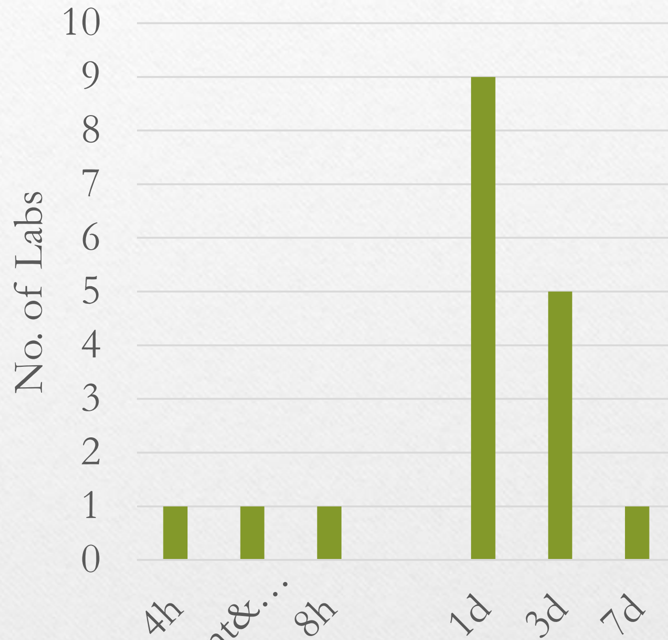
Section 2 - **Operation and Analysis**

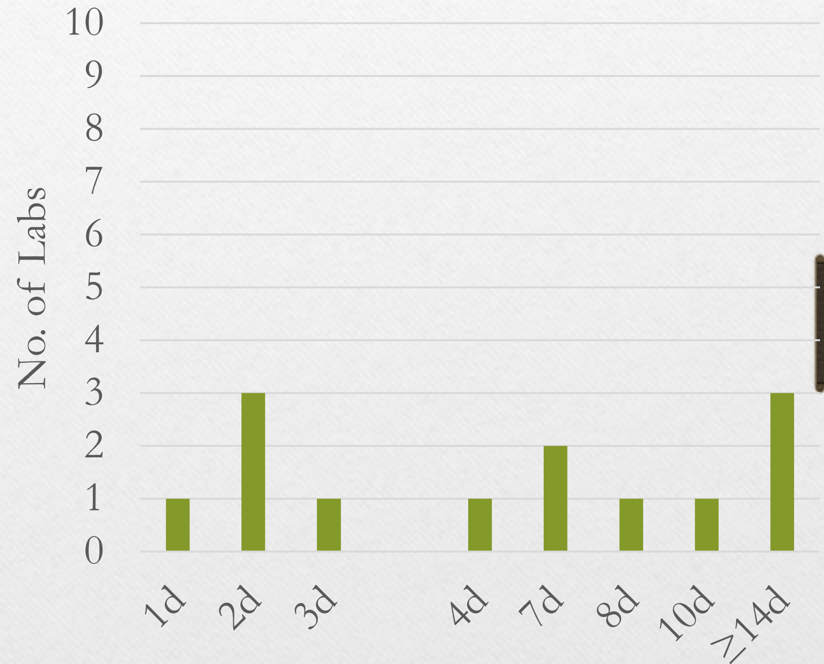
Q-B1-3: Analytical platforms

Total PRL	Analytical range	Hook Effects
Roche (n=12)	1 – 10,000 (100,000 with 1/10 dilution)	270,000
Abbott (n=3)	4200 (42,000 with 1/10 dilution)	
ADVIA Centaur (n=2)	**6.4 – 4240** (21,000 with 1/5 dilution)	636,000
Beckman (n=1)	**1 – 4240** (42,400 with 1/10 dilution)	636,000
Siemens (n=2)	6.36 – 4240 (21,000 with 1/5 dilution)	636,000
Referral to other labs (n=2)		

Recommend: Reporting units: mU/L or miu/L

Q-B4-6: TAT





MPRL assay running frequency	No. of labs
Daily in batch	5
2-3 x a week	7
Weekly batch	6

Q-B7 Assay protocol - Literature

[**Beltran L, 2008**]

- 250 uL of sera, + 250 uL 25% PEG 6000 (250 g/L in PBS)
- Incubated for 10 min at RT
- Centrifuge at 14 000g for 5 min
- The 25% (wt/vol) stock PEG solution was stored at 4 °C for no more than 14 days and allowed equilibrate to room temperature before use.

[Fahie-Wilson MN. 1993]

- Centrifugation at 1500g for 30 min

Q-B7a Assay protocol

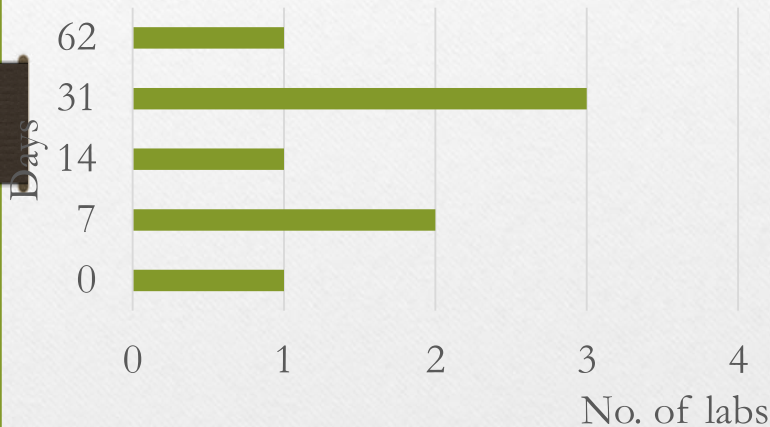
Minimum sample volume	**200 – 1000 ul**
Sample volume added to equal volume of PEG	140 – 250 ul
Paired sample + diluent	7 labs do not perform 11 labs perform
Incubation time for sample/PEG mixture	1 lab : 10 min Rest: none

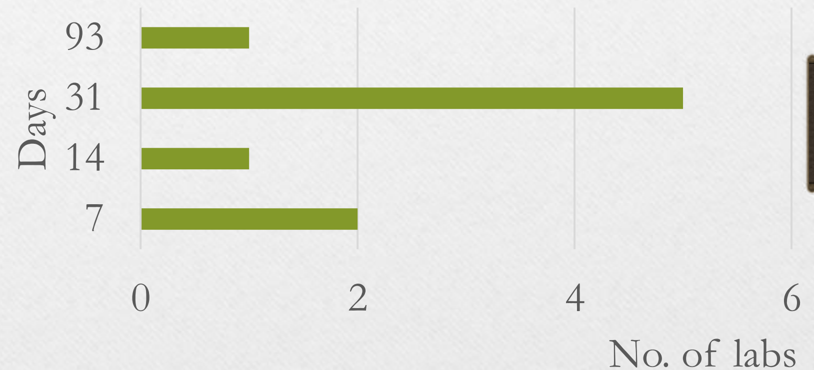
	Centrifugation speed		
Time	1400g-1800g	3000-4000 rpm	10000-14000 rpm
5 min	1		5
10 min		3	
15 min			1
20 min		1	2
30 min	4	2	

Recommend: Consider introducing paired sample + diluent in to the protocol if not running duplicates. Consider shortening the length of centrifugation to speed up the MPRL assay protocol and save staff time.

Q-B7c PEG

PEG working solution	25% PEG6000 used in all labs	
PEG dissolved in	Deionised water: 16 labs	Diluent/PBS: 2 labs





Recommend: dissolve PEG6000 in diluent. Store the PEGs solution at 4oC in aliquots prior to use. Verified length of storage was up to 4 weeks, but this might vary depending different analytical platforms. Labs should verify the PEG stability locally. PEG solution should return to RT prior use.

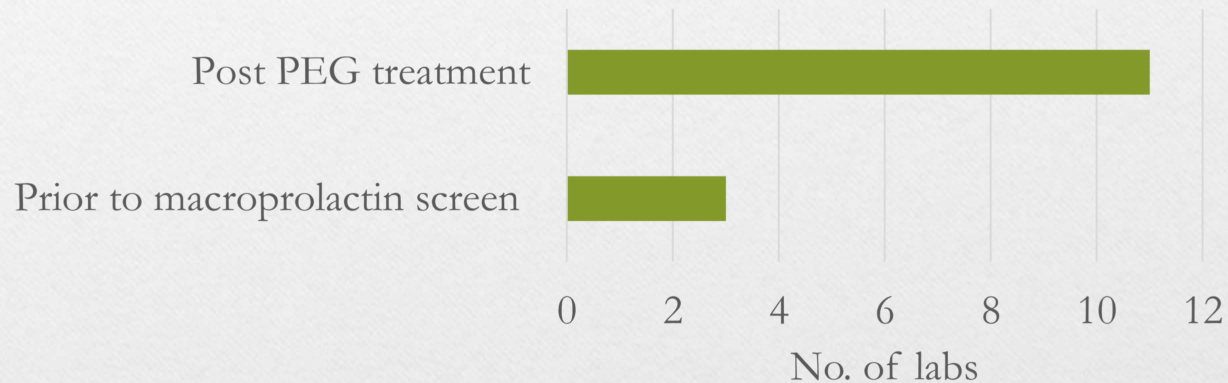
Q-B8a Calculations

		No. of labs
Estimated BMP	Sample PEG x dilution factor 2	9
	Sample PEG x dilution factor 2.6 or 2.7	3 &1
	BMP not reported	2
MPRL Recovery	Sample PEG /sample diluent x 100%	10
	BMP/total prolactin x 100%	6
	[100 (2z x 1.163)] / y Where z= prolactin post PEG ppt ; y= original prolactin result	1

Recommend: If use a dilution factor of 2.6 instead of 2 for correcting the recovery of BMP, the RR for BMP should the same as the total prolactin.

Q-B7: Case Study

When the original prolactin result in the sample is above the upper limit of assay range on a neat sample (e.g. a prolactin of 5000):



Q-B10: IQC

	No. of labs
Previous patient samples Neg or Pos	4
Commercial IQC ○ Liquichek Immunoassay Level 3 (Biorad) ○ Immunoassay level 3 IQC (Randox) ○ Multichem IA (IA300X) (Technopath) +ve ○ Lyphochek level 2, Immunoassay plus control (Biorad) -ve	7
Both patients and commercial IQC	5

Recommend: To include samples with one high and one low recovery as QC control in each batch. Use commercial IQC with a derived target value.

Suggested MPRL Assay acceptance criteria
Acceptable IQC values for PRL assay
Recovery check: acceptable percent recovery for MPRL IQC & don’t accept % recovery > 110 %
Dilution check: diluted prolactin result x2 must be acceptable +/- 20 %, when compared with original prolactin level

Q-B11-12: EQA

	No. of labs
UKNEQAS Edinburgh Peptide hormones (However, samples with raised PRL and low % are rare)	14
Sample swap with other labs	3
Precision test with each batch of samples using a pooled patient sample	1

Is MPR in scope for UKAS	No. of labs
Yes	14
No	1
Not stated	2

Recommend: consider organising sample swaps with other labs where samples with significant raised PRL and low % could be sourced.

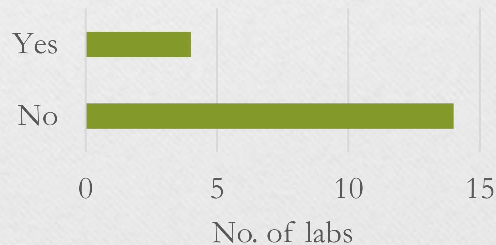
Part 3. Macroprolactin - MPRL

Section C - **Interpretation and Reporting**

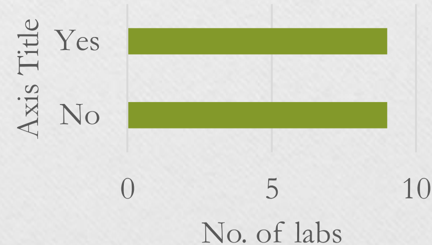
Q-C1: What is reported?

when -ve	when +ve	Equivocal	No. of labs
≥60%	<60%	n/a	7
≥40%	<40%	n/a	2
≥56%	<56%	n/a	1
60	40	40-60%	4
50	40	40-50%	1
≥60%	<56%	56-60%	1

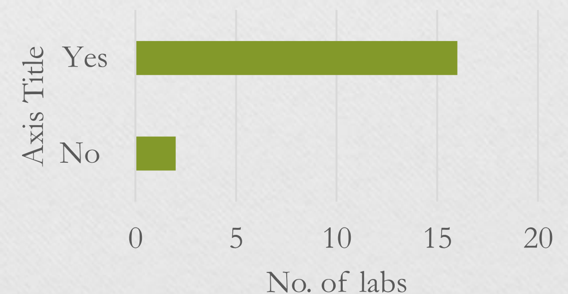
When -VE MPRL recovery, will % recovery and BMP reported?



When +VE MPRL recovery, will % recovery reported?



When +VE MPRL recovery, will BMP reported?



Q-C2: RR for PRL and BMP

	Total PRL	No. of labs	Reference	BMP	No. of labs	Reference
Roche (n=12)	F:102-496; M:86-324	10	Kit insert	F:75-381; M:63-245	5	Beltran L, 2008
	F:<500; M:<450	1	?? source	F&M :<430	1	?? source
	F&M :0-500	1	->plan to change	F&M :<520	1	?? source
Abbott (n=3)	?? source	1	Kit insert		1	
	*F:pre&post-menopausal 71 – 566 &58 – 416; M:60-300	1	From Abbott, internally verified	F:<469; M:<301	1	?? source
	F:<1000; M:<700	1	Clinical relevance	F:<347; M:<229	1	Beltran L, 2008
ADVIA Centaur (n=2)	F:59-619; M:45-375	1	Kit insert	Same as total PRL	1	a dilution factor of 2.6 applied to BMP
	F: pre&post-menopausal 59-619 &38-430 ; M:45-375	1	Kit insert	F&M:70-438 (**higher than total PRL)	1	Jassam NF 2009
Beckman (n=1)	*F:pre&post-menopausal 71 – 566 &58 – 416; M:<278	1	Kit insert	F:<469; M:<301 (**higher than total PRL)	1	Locally derived

Q-C2: Literatures for reference ranges

Table 2. Reference intervals for total prolactin (mIU/L) in serum samples from males and females for each immunoassay platform.

Method	Parametric lower	Estimate upper	Manufacturer's range
Samples from males			
Access	58	277	56–278
Centaur	63	262	45–375
Immulite	70	281	53–360
Elecsys	72	331	86–324
Architect	85	310	54–381
AIA	89	365	97–440
Samples from females			
Centaur	71	348	59–619
Immulite	75	396	40–530
Access	77	408	71–566
Elecsys	88	492	102–496
Architect	98	447	25–629
AIA	105	548	111–780

Table 3. Parametric reference intervals for post-PEG prolactin (mIU/L) in male and female sera for each immunoassay platform.

	Male range		Female range	
Analyzer	Lower	Upper	Lower	Upper
Centaur	61	196	66	278
Elecsys	63	245	75	381
Access	70	301	92	469
Architect	72	229	79	347
AIA	73	247	83	383
Immulite	78	263	85	394

(taken from Beltran L, et al 2008)

Table 1 Reference intervals for total prolactin and post-PEG prolactin (mIU/L) for Advia Centaur

	Monomeric reference interval (mIU/L)	Total prolactin reference interval (mIU/L)
Men and women (this study)	70–438	70–538
Women only (this study)	74–413	74–505
Women (Beltran *et al.*)	66–278	61–404

(taken from Jassam NF et al. 2009)

Recommend: labs to review their RR in use. Same analytical platform should harmonise their RR according to the literature. Source of the RR for total and BMP should be consistent.

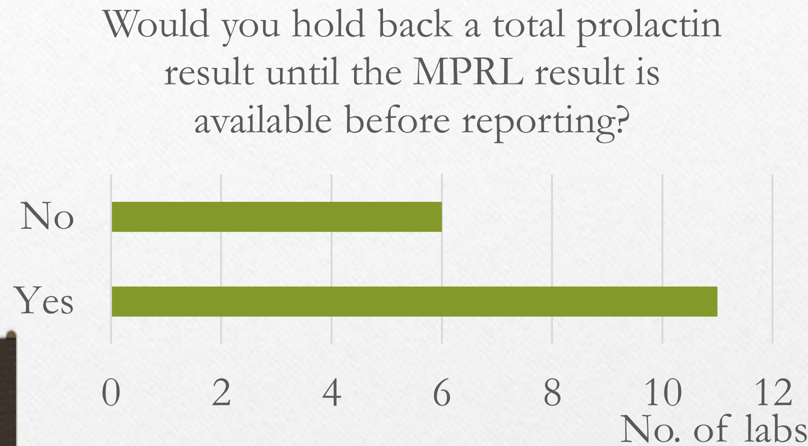
Q-C3: Interpretive comments

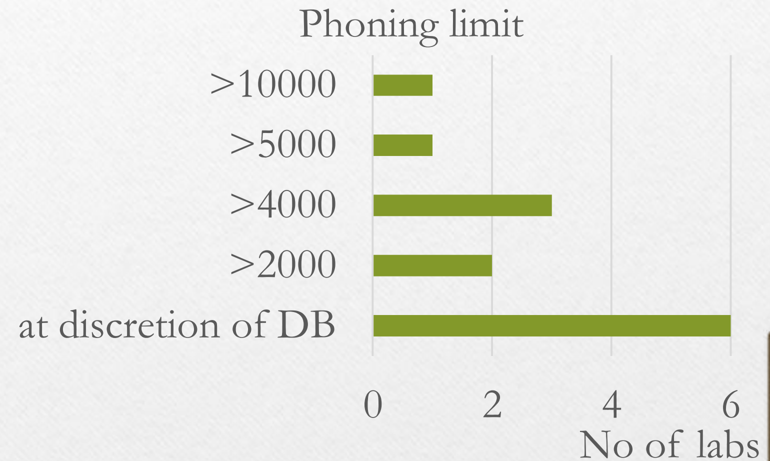
when –ve for MPRL	
New patients with a PRL ≥ gender specific decision cut off	Elevated prolactin concentration. Interpret the prolactin in light of clinical details and drug therapy. Exclude dopamine antagonists (such as anti-psychotics, SSRIs and anti-emetics e.g. metoclopramide), stress, pregnancy, lactation, hypothyroidism and renal failure. Suggest rule out drug effects and repeat to confirm. The Duty Biochemist is available to discuss these results on xxxx
if significantly raised	Significantly raised prolactin concentration. Prolactinoma is a possibility, but other causes should be excluded including dopamine antagonists (such as anti-psychotics, SSRIs and anti-emetics eg metoclopramide), stress pregnancy, lactation, hypothyroidism and renal failure. Suggest rule out drug effects and repeat to confirm. The Duty Biochemist is available to discuss these results on xxxx

Q-C3: interpretive comments

when +ve for MPRL	
New patients with a BMP ≤ the gender specific decision cut off.	Normal bioactive monomeric prolactin (i.e. physiologically active prolactin), despite raised total prolactin. No further investigation required. Note: The serum has been tested for the presence of macroprolactin, which is a non-pathological physiologically inactive form of prolactin that causes a falsely high total prolactin result. The Duty Biochemist is available to discuss these results on xxxx
New patients with a BMP ≥ the gender specific decision cut off.	Raised total prolactin concentration. Raised bioactive monomeric prolactin (i.e. physiologically active prolactin), showing TRUE HYPERPROLACTINAEMIA. Interpret the prolactin in light of clinical details and drug therapy. Exclude dopamine antagonists (such as anti-psychotics, SSRIs and anti-emetics e.g. metoclopramide), stress, pregnancy, lactation, hypothyroidism and renal failure. Suggest repeat to confirm. Note: The serum has been tested for the presence of macroprolactin, which is a non-pathological, physiologically inactive form of prolactin that causes a falsely high total prolactin result. The Duty Biochemist is available to discuss these results on xxx if required.

Q-C4 Authorising and phoning PRL results





Recommend:

- Consider hold back the PRL result if MPRL TAT is short, patient was asympomatic and the initial PRL result was not significantly raised.
- If the initial prolactin result is grossly elevated or there is an indication that the patient is symptomatic such as visual field disturbances, the result should be authorised and phoned out to the requesting clinician with the caveat that it is still awaiting a macroprolactin screen.
- Prolactin results ≥10,000 mU/L should be telephoned immediately without waiting for PEG precipitation.

Q-C5 Refer MPRL for confirmation

1 Lab	Used to send samples with recovery of 40-60% for confirmation by GFC (no longer routinely available at Addenbrookes's). Now send samples within network using a different method that is less susceptible to macroprolactin interference than Roche method
1 Lab	By a different method- Abbott, Beckman , Centaur (and PEG) as Centaur has reportedly the lowest cross reactivity , then GFC if clinical
1 Lab	During validation some specimen were sent to Southend for gel filtration. Since that time some specimens sent to Cambridge for analysis on Centaur platform. No interferences identified
1 Lab	Samples have previously been sent to Southend Hospital for analysis using Tosoh AIA. Historical validation identified no false positive results.

Q-C6: Case Study

- **A sample from a 16 y/o girl whose prolactin was initially about 1500 mu/L with only about 15% recovery after PEG. Her monomeric prolactin was within the reference range for your local assay. The clinical details given were Galactorrhoea. What would you do?**

-Repeat the sample to confirm 1500 mu/L is genuine.
-Rule out any hook-effect with sample dilution
-Discuss with GP, add on hCG, TFTs and U&E
-Refer MPRL for conformation
-Suggest refer to endocrine if results not indicative of cause

Q-C7: Trust guideline

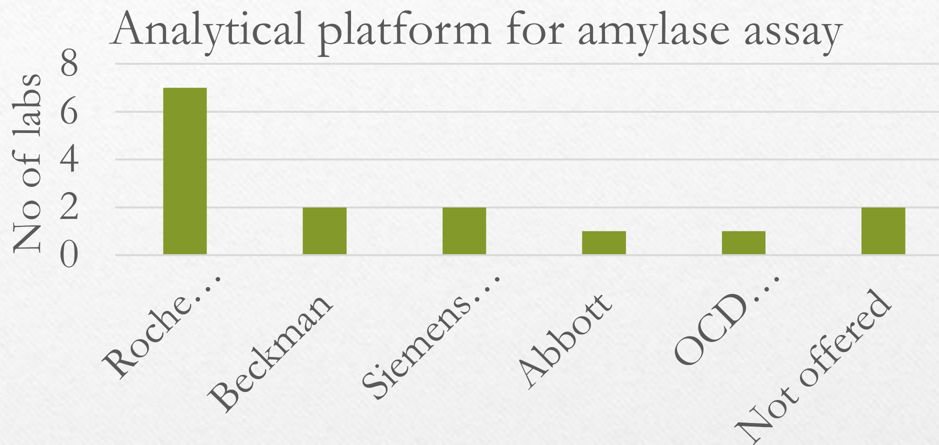
- Imperial Endocrine Bible (http://www.imperialendo.com/for-doctors/endocrine-bible)
- Prolactin and antipsychotic drug therapy: guidelines for management of hyperprolactinaemia in adults https://www.ekhuft.nhs.uk/patients-and-visitors/services/pathology/clinical-biochemistry/guidelines/

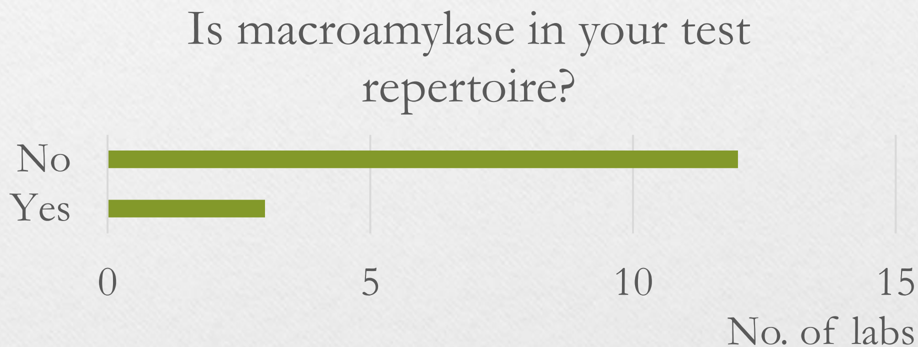
Recommend:

- Develop trust guidelines for the investigation of raised prolactin, in particular for patients who are on medications which cause the greatest degree of elevation of PRL.

Part 4. Macroamylase

Q-1-2: Analysis





Alternatives:

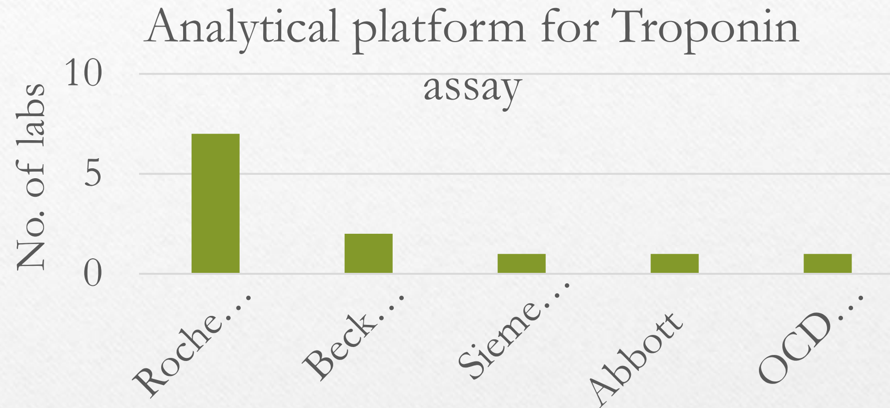
- Lipase
- Amylase isoenzymes

(GOSH-Stopped offering in April 2020)

- Urine amylase

Part 5. Macrotroponin

Q-1-2: Analysis

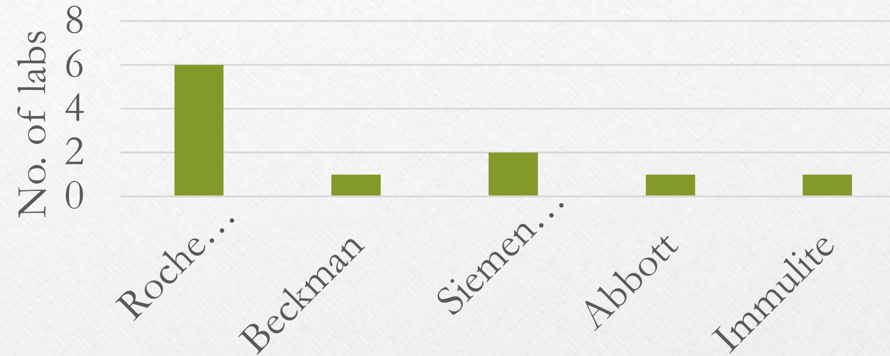


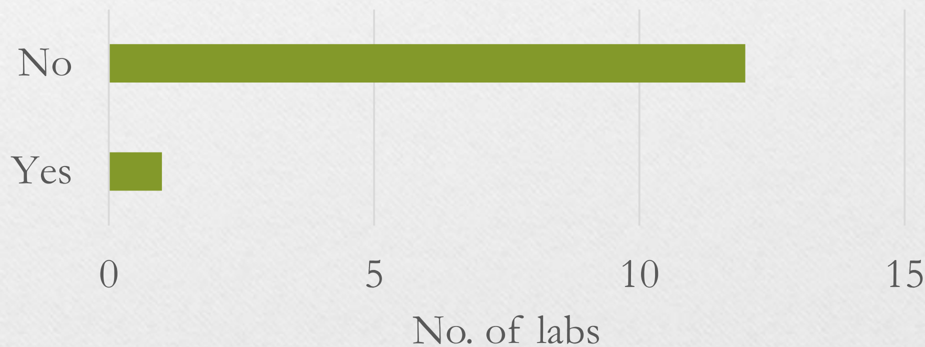
Alternatives:

- Dilution study
- by alternative method elsewhere to exclude interference
- could do PEG precipitation method in house (using MPRL protocol)
- CK and CK-MB

Part 6. MacroTSH

Q-1-2: Analysis





Alternatives:

- Dilution study
- Send for measurement by alternative method
- If still raised, send for Thyroid panel at Addenbrookes

References

Beltran L, el all. Serum total protein and monomeric prolactin reference intervals determined by precipitation with polyethylene glycol: Evaluation and validation on common immunoassay platforms. Clin Chem 2008; 54: 1673-1681

Fahie-Wilson MN, Brunsden, Surry J et al.
Macroprolactin and the Roche Elecsys Prolactin assay: Characteristics of the reaction and detection by precipitation with polyethylene glycol.

N F Jassam, A Paterson, C Lippiatt, J H Barth (2009) Macroprolactin on the Advia Centaur: Experience With 409 Patients Over a Three-Year Period. Ann Clin Biochem. 2009 Nov;46(Pt 6):501-4.