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Intraoperative PTH (ioPTH)

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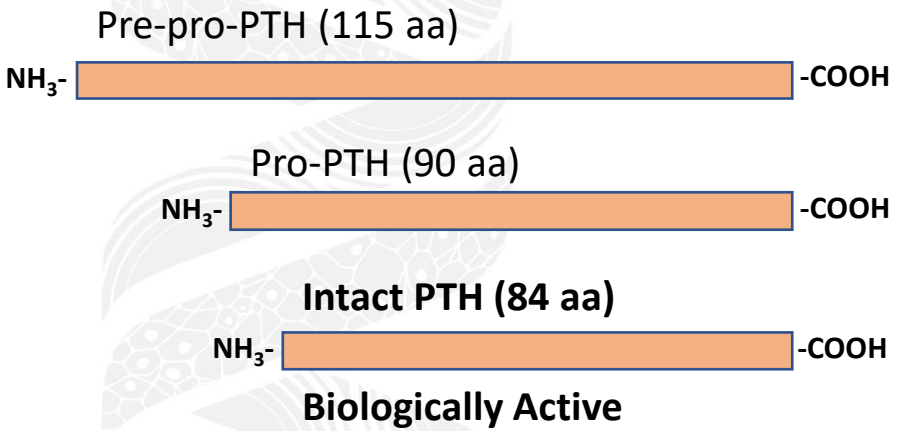
Summary

- Parathyroid hormone (PTH)
  - Synthesis, metabolism and regulation
- Primary Hyperparathyroidism (PHPT) – NG132
- Surgery for PHPT
  - Imaging
  - Biochemistry - ioPTH
    - Near Patient Testing (NPT)
    - Point of Care Testing (POCT)

PTH synthesis & metabolism

- Peptide hormone encoded by gene on short arm of chromosome 11
- Synthesised in the chief cells of the parathyroid glands



- Metabolised mainly in the liver and kidney into biologically active N-terminal fragment (1-36 aa) and inactive C-terminal fragment (37-84 aa), but also mid-fragments

- **Intact PTH** and N-terminal PTH– short half-life ~5 min
- Inactive C-terminal fragment – half-life ~30-40 min
- Non-(1-84) or long C-terminal fragment increased in CKD – long half-life

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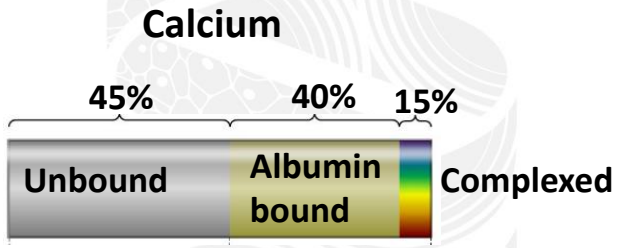
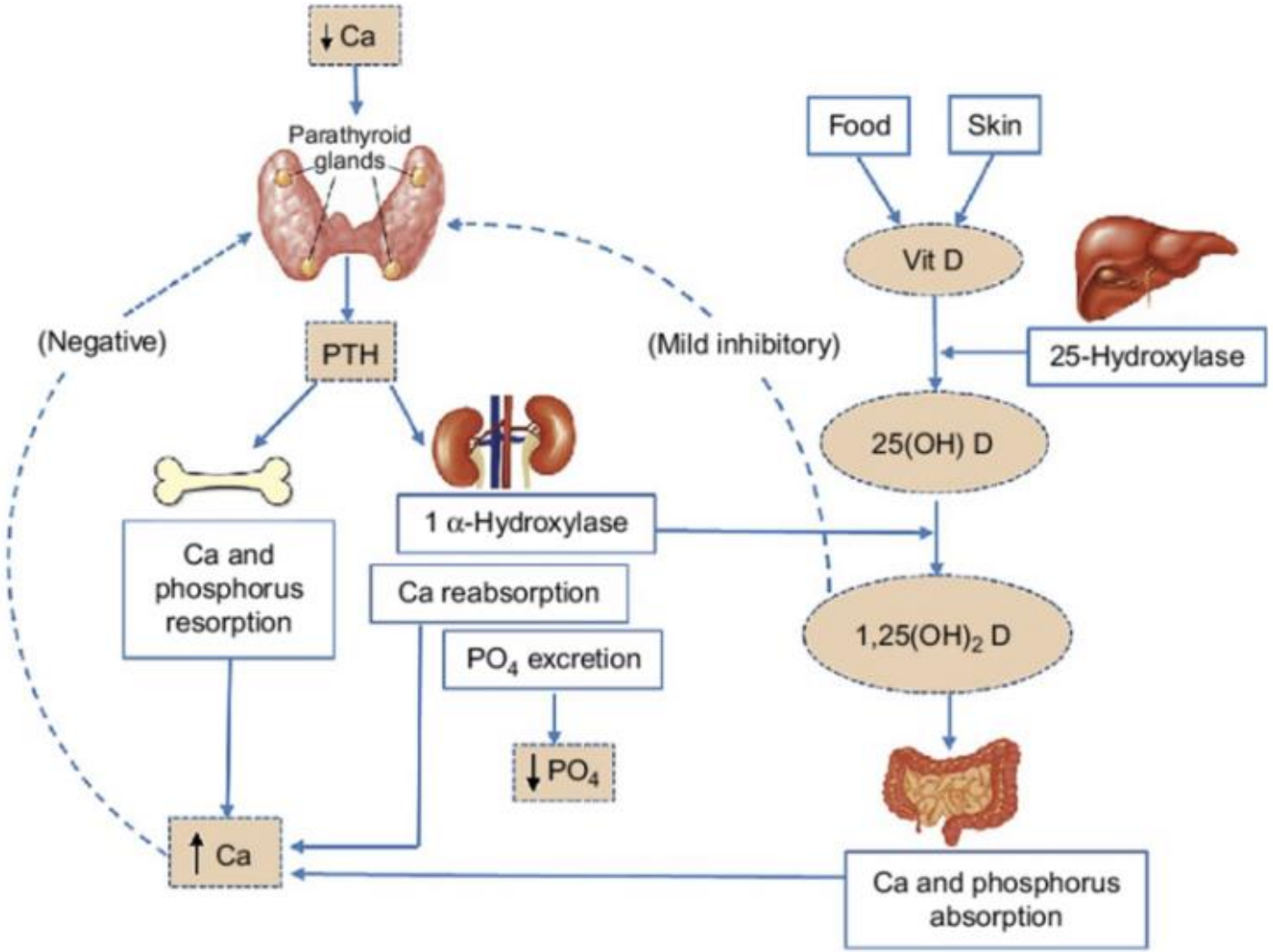
PTH regulation

- Hypocalcaemia triggers PTH secretion from the parathyroid glands



- Direct effect on kidneys and bones, indirect effect in the gut.
- Increased Ca^{+2} - Negative feedback to parathyroid glands



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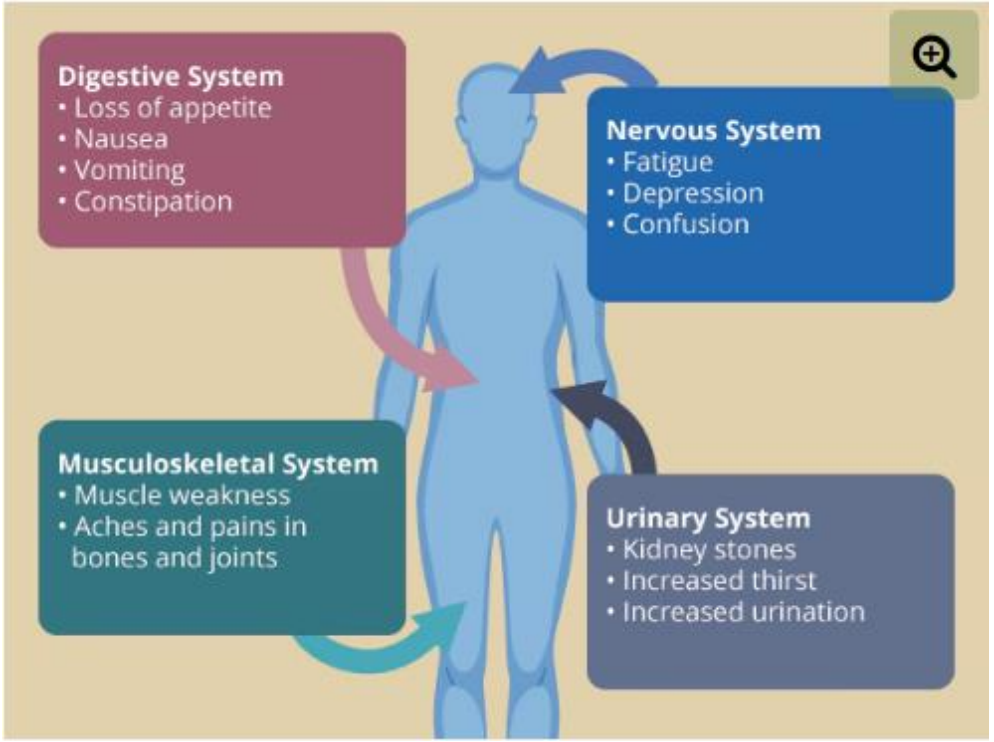
Hyperparathyroidism

Primary HPT

- Disorder of the parathyroid glands
- Classic PHPT: “Stones, bones, groans and psychiatric overtones”
- Asymptomatic PHPT: no classical symptoms, weakness, high fatigability. Loss of cortical bone and/or lower BMD common finding
- Women more commonly affected than men (3:1) – predominantly post-menopause
- Increasing prevalence, 3rd most common endocrine disorder after diabetes and thyroid diseases
- Cure: parathyroidectomy – doubled in NHS between 2000 and 2010



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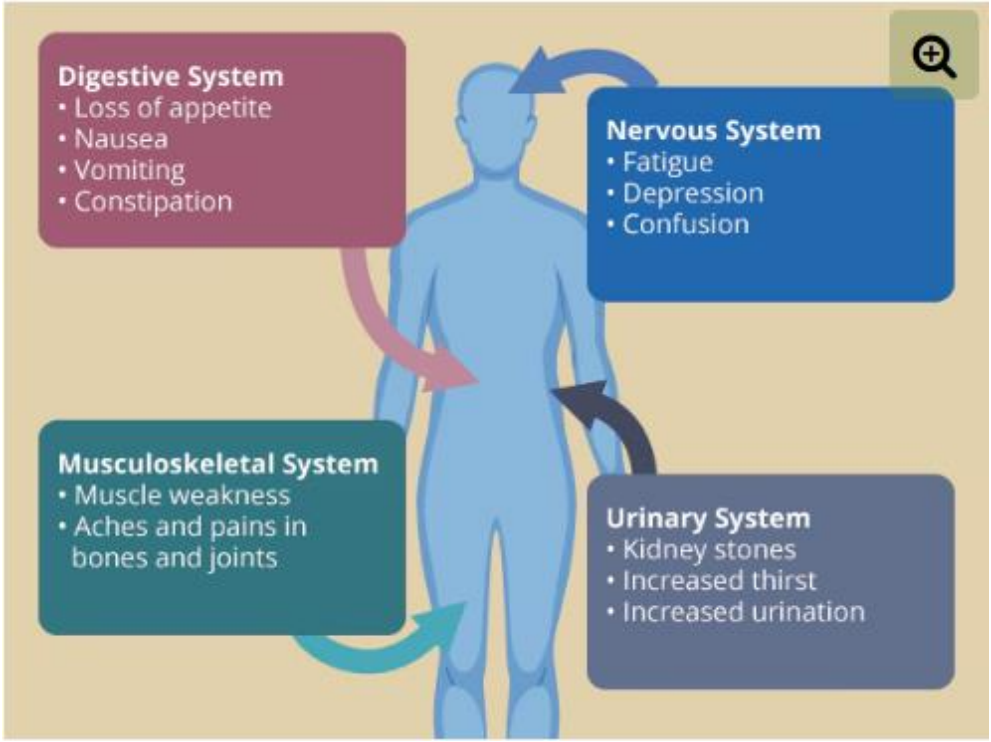
Hyperparathyroidism

Secondary HPT

- Physiological response of the parathyroid glands to underlying causes of hypocalcaemia
- VitD deficiency, malabsorption, low Ca intake, CKD

Tertiary HPT

- Prolonged untreated secondary HPT in advanced CKD
- Autonomous PTH hyper-secretion
- Can lead to kidney allograft rejection



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NG132 (May 2019) – PHPT diagnosis & management

Primary Care:

adj.Ca^{+2} ≥ 2.60 mmol/L

or

adj.Ca^{+2} ≥ 2.50 mmol/L
+
Symptoms

PTH*

Not suppressed
+ ↑adj.Ca^{+2}

> mid-Ref. Range
+ symptoms

Probable diagnosis of PHPT

Refer to **Secondary Care**

*** PTH Stability at Room Temp**

- Whole blood EDTA – 24h
- Clotted whole blood – 3h

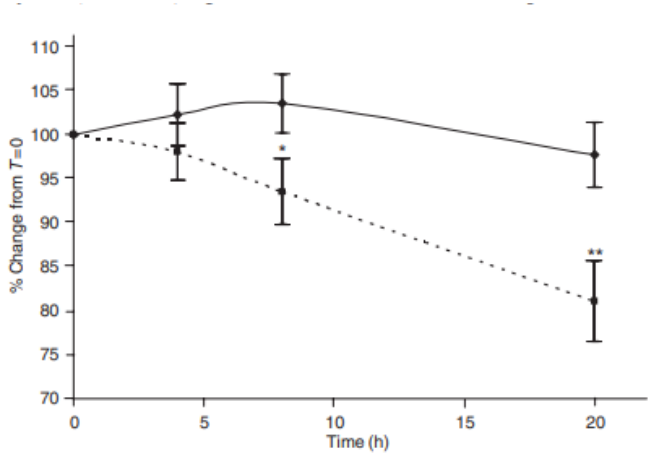

Figure 1 *Change in parathyroid hormone over time in EDTA (solid line) and serum separator tubes (dashed line) showing a significant decrease in serum separator tubes (*P = 0.0099, **P = 0.0004) but not in EDTA tubes. Error bars are the 95% confidence intervals in each case*

Ann Clin Biochem 2007; **44**: 297-299

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NG132 (May 2019) – PHPT diagnosis & management

Secondary Care:

- Measure VitD, & supplement if needed
- Exclude drug induce PHPT (thiazides, lithium)
- Assess renal function
- Familial Hypocalciuric Hypercalcaemia (FHH) – Calcium/Creatinine excretion ratio <0.01 on normal Ca^{+2} diet

→

- Mutation in CASR gene
- Patients asymptomatic
- Do NOT benefit from parathyroidectomy

- DXA of lumbar spine, distal radius and hip

Referral for surgery

Preoperative Imaging
- Ultrasound
- and/or
- Scintigraphy

→

- Bilateral Neck Exploration (BNE)
- OR
- Minimal Invasive Parathyroidectomy (MIP)

Do NOT use ioPTH monitoring in first-time parathyroid surgery

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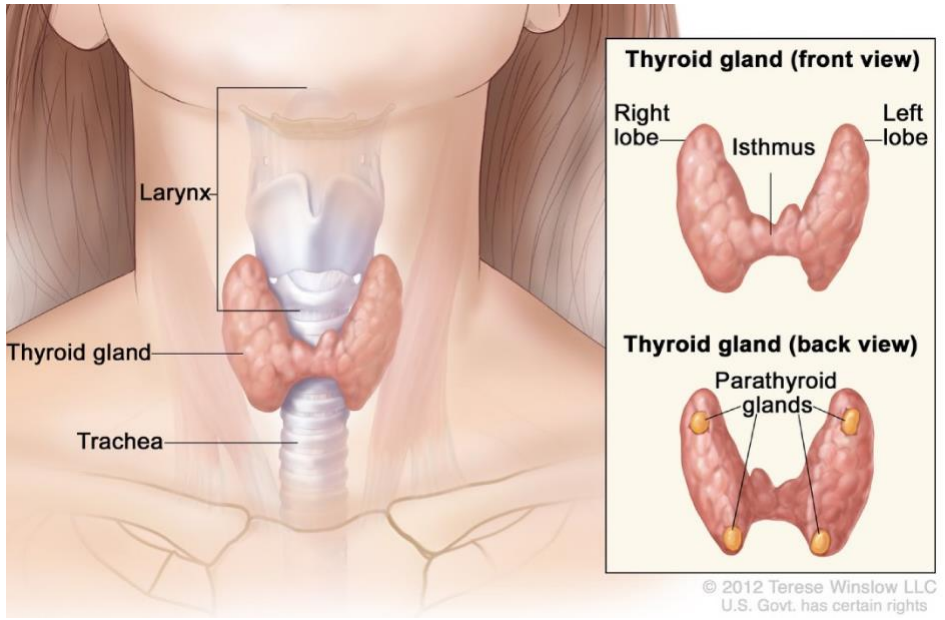
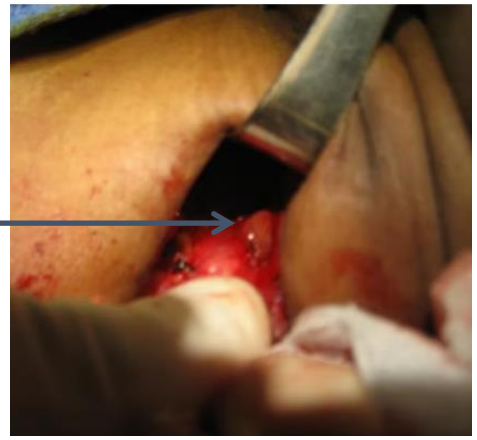
Parathyroid Glands

- Pea-size glands – vast majority of people have 4
- Located posteriorly to the thyroid
- **PHPT**
  - Single adenoma 85%
  - Multiple gland disease (MGD) and hyperplasia 15-20%
  - Carcinoma ~1%

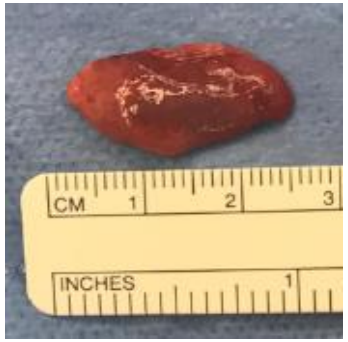


- Majority of cases sporadic
- Hereditary syndromes like MEN1 & MEN2A associated with MGD and/or hyperplasia

Healthy parathyroid gland



Enlarged parathyroid gland



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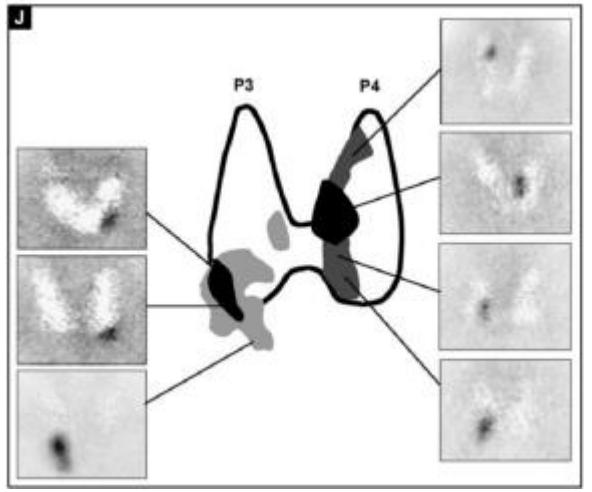
Imaging

- **Purpose**: localisation of hypersecreting glands entopic/ectopic
- **Ultrasound (US)**
  - Structural features, no radiation, high resolution, relatively cheap
  - Lymph nodes also small and echogenic
- **Scintigraphy**
  - Single-photon scintigraphy with 99mTc-Sestamibi, or dual tracer 99mTc-pertechnetate and 99mTc-sestamibi
  - Uptake related to number & activity of mitochondria in oxyphil parathyroid cells. No uptake from the chief cells.
- Concordant US and MIBI identify accurately 95% of single adenomas
- **Only 52 -64% of US & MIBI are concordant**



Morris MA *et al*. Parathyroid Imaging: Past, Present and Future

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Role of ioPTH

- MIP favoured surgical approach over BNE
  - Similar cure rate to BNE for sporadic single gland disease
  - Smaller incision
  - Shorter operation time

- Bilateral internal jugular venous sampling– alternative for lateralisation of affected gland
- PTH has a short half-life (~5min) → criteria developed based on % drop post excision of the affected gland(s)

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Role of ioPTH

Criterion			Cure
Hale	PTH falls to lower end of Ref.Range by 15 min post-excision		Adjusted Calcium within reference range for at least 6 months post-surgery
Rome	PTH ≥ 50% fall of pre-excision value	PTH within Ref.Range 20 min post-excision or PTH 0.8 pmol/L lower than PTH at 10 min post-excision	
Vienna	PTH ≥ 50% fall from pre-incision value at 10 min post-excision		
Miami	PTH ≥ 50% fall from pre-incision or pre-excision value (whichever is the highest) at 10 min post-excision		

- Miami criterion developed and optimised in the 1990s
  - Highest overall accuracy (93 – 97%) - Most widely used

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UCLH – imaging, ioPTH & Miami Criterion since 2006

- Ultrasound and single tracer dual phase Tc-99 m MIBI SPECT/CT
  - **Concordant**: both agree on lateralisation of adenoma
  - **Discordant type 1**: adenoma identified only in 1 scan
  - **Discordant type 2**: US and MIBI contradicting each other
  - **Negative**: no adenoma identified on scan
- STAT-IntraOperative-Intact-PTH Immunoassay Kit (STAT-IO-I-PTH) future diagnostics

TABLE 4 Cure rates and IOPTH monitoring added value

Subgroup	N	Cure rate (N, %)			
		IOPTH	No IOPTH	IOPTH add value	Sig
Overall	617	603 (97.7%)	517 (83.7%)	86 (14%)	$P < 0.05$
Concordant	393 (63.7%)	385 (98%)	339 (86.3%)	46 (11.7%)	$P < 0.05$
Discordant (type 1)	136 (22%)	134 (98.5%)	119 (87.5%)	15 (11%)	$P < 0.05$
Discordant (type 2)	42 (6.8%)	41 (97.6%)	27 (64.3%)	14 (33%)	$P < 0.05$
Negative	46 (7.5%)	43 (93.5%)	32 (69.5%)	11 (24%)	$P < 0.05$

Surgeries for PHPT 2006 – 2016

Shawky M. *et al*. Impact of intraoperative parathyroid hormone monitoring on the management of patients with primary hyperparathyroidism

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UCLH – imaging, ioPTH & Miami Criterion

TABLE 2 Performance of imaging and IOPTH monitoring

	US			MIBI			IOPTH		
Measure	SGD	MGD	Overall	SGD	MGD	Overall	SGD	MGD	Overall
Accuracy	89	60.8	84.9	86.6	52.8	81.5	98.7	89	97.2

IOPTH, intraoperative parathyroid hormone; MGD, multi gland disease; MIBI, methoxy iso butyl isonitrile; SGD, single gland disease; US, ultrasound.

TABLE 4 Cure rates and IOPTH monitoring added value

		Cure rate (N, %)					
Subgroup	N	IOPTH		No IOPTH		IOPTH add value	Sig
Overall	617	603 (97.7%)		517 (83.7%)		86 (14%)	$P < 0.05$
Started as MIP	477 (77.3%)	468 (98.1%)	$P > 0.05$	416 (87.2%)	$P < 0.05$	52 (11%)	$P < 0.05$
Started as BNE	130 (21%)	126 (96.9%)		94 (72.3%)		32 (24.6%)	$P < 0.05$
TA	10 (0.7%)	9		7		2 (20%)	$P > 0.05$
Initial	571 (92.5%)	559 (97.9%)	$P > 0.05$	488 (85.5)	$P < 0.05$	71 (12.4%)	$P < 0.05$
Reoperative	46 (7.5%)	44 (95.7%)		29 (63)		15 (32.6%)	$P < 0.05$

BNE, bilateral neck exploration; IOPTH, intraoperative parathyroid hormone; MIP, minimally invasive parathyroidectomy; TA, thoracic approach.

Shawky M. *et al*. Impact of intraoperative parathyroid hormone monitoring on the management of patients with primary hyperparathyroidism

Surgeries for PHPT 2006 – 2016

- Limitations of imaging
- Added value of ioPTH
- Possibility to minimise preoperative scans

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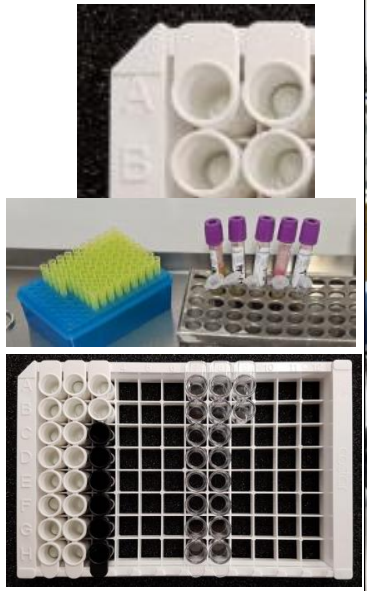
UCLH – Near Patient Testing (STAT-IO-I-PTH)

- Chemiluminescent immunometric assay – intact PTH in EDTA plasma
- 2 goat polyclonal antibodies
- C-terminal anti-PTH on coated on well-surface
- N-terminal anti-PTH labelled with isoluminol & lyophylised in the form of accusphere
- Incubation for 5 min followed by wash of excess/unbound antibody
- Addition of NaOH and H_2O_2 generates light – signal proportional to the concentration of PTH in sample.
- Samples always analysed in **duplicate** – mean & %CV reported
- Total **TAT ~10 min**



UCLH – Point Of Care Testing

- Chemiluminescent immunometric assay – intact PTH in whole blood EDTA
- 2 goat polyclonal antibodies
- One Ab coated on paramagnetic particles
- One Ab conjugated to with ALP
- Cartridge based
- Incubation is followed by a wash step
- Addition of ALP substrate generates light – signal proportional to the concentration of PTH in sample.
- **Single** measurements
- Total **TAT ~5 min**

Precision

	Manufacturer's data (NBCL)		
NBCL quality controls	n	Concentration (pmol/L)	% CV
Intra-assay variability	33	6.3	8.5
	31	30.9	6.2
Inter-assay variability	33	6.3	11.6
	31	30.9	10.0

	UCLH's data (NBCL)		
NBCL quality controls	n	Concentration (pmol/L)	% CV
Inter-assay variability	12	5.6	14.3
	12	30.6	15.0

	UCLH's data (NBCL)		
BioRad quality controls	n	Concentration (pmol/L)	% CV
Inter-assay variability	25	5.8	14.4
	25	39.9	5.1
	25	131.8	5.1

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Comparison of NBCL, FD and Roche

PTH	Roche Elecsys	future diagnostics	nbcl
Immunoassay	2nd generation/intact PTH	2nd generation/intact PTH	2nd generation/intact PTH
Cross reactivity with long C-fragment (7-84)	99%	Not stated, however N-terminal Ab targets PTH from position 7 onwards	Not stated, however N-terminal Ab targets PTH from position 7 onwards
Sample Type	**Plasma** EDTA	**Plasma** EDTA	**Whole Blood** EDTA
Antibodies	**Mouse monoclonal** (biotinylated/ruthenium complex)	**Goat polyclonal** (isoluminol oxidised in presence of H_2O_2 in NaOH)	**Goat polyclonal** (ALP plus substrate)
Traceability	**WHO 95/646** (recombinant human PTH) Recovery 100% ± 4%	**WHO 79/500** (human PTH) Recovery 51%	**WHO 95/646** (recombinant human PTH) Recovery 100.4%
Detection	electrochemiluminescence	chemiluminescence	chemiluminescence

Comparison of Roche, FD, NBCL

UK NEQAS [Edinburgh]

UK NEQAS for PTH, ACTH and hCT

Distribution : **204** Date : 05-Nov-2024

Analyte : PTH (pmol/L)

	P798			P799			P800		
	n	Mean	GCV	Outl.	Mean	GCV	Outl.	Mean	GCV	Outl
All methods	310	63.9	26.5	2	184.5	22.9	2	4.7	21.4	4
PTH Methods 1	285	64.4	25.5	1	185.8	21.8	2	4.7	20.1	4
Abbott Alinity	48	78.9	8.6	0	222.5	6.3	0	6.5	6.6	0
Abbott Architect	16	79.8	6.8	0	225.6	4.6	0	6.8	5.0	0
Beckman Access	32	66.8	9.9	1	197.5	6.7	1	4.4	6.4	1
1 or 2	5	67.3	9.7	0	192.1	10.0	0	4.4	7.0	0
DxI	27	66.7	9.8	1	198.4	6.7	1	4.4	6.8	1
OCD (J&J) VITROS	2	89.8		0	258.7		0	6.0		0
Roche Elecsys	142	54.1	7.1	0	161.2	4.1	1	4.3	4.1	3
1010, 2010, e411	4	56.1		0	167.7		0	4.4		0
Cobas PURE e402	4	54.0		0	164.0		0	4.3		0
E170, e601, e602, e801	129	54.1	7.1	0	160.8	4.1	1	4.3	4.2	3
Siemens A Centaur	6	77.3	2.2	0	227.9		0	4.2	6.7	0
Siemens Atellica	38	81.8	7.4	0	254.6	5.5	0	4.5	4.8	0
Tosoh AIA	1	72.7		0	211.5		0	4.1		0
PTH Methods 2	17	45.3	14.5	1	130.4	10.5	0	3.4	24.2	0
DS Liaison 1-84 PTH	5	46.3	26.0	0	144.2	9.4	0	2.6	5.5	0
Fujirebio Lumipulse	1	41.7		0	114.6		0	2.5		0
G1200	1	41.7		0	114.6		0	2.5		0
Roche Elecsys (Bio)	11	45.0	13.0	1	125.7	5.1	0	3.8	5.6	0
E170, e601, e602, e801	10	44.5	12.8	1	124.7	4.2	0	3.8	4.5	0
PTH STAT Methods	8	115.1	80.6	0	347.7	84.1	0	8.5	45.1	0
Future Diag STAT	5	159.8	18.2	0	467.6	35.6	0	10.6	4.8	0
NBCL CONNECT	3	69.5		0	204.7		0	6.7		0

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UK NEQAS [Edinburgh]

UK NEQAS for PTH, ACTH and hCT

Distribution : **204** Date : 05-Nov-2024

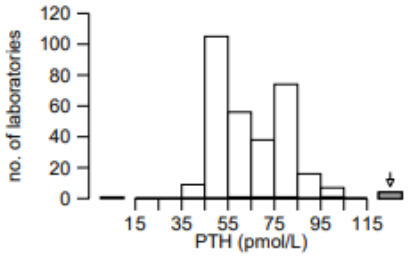
Analyte : PTH (pmol/L)

Spec.	Pool	Pool description
P798	UP069	Purified PTH (1-84) in EDTA plasma.
P799	UP079	Purified PTH (1-84) in EDTA plasma.
P800	UP082	Base pool of EDTA plasma.

All methods
PTH STAT Methods
Future Diag STAT

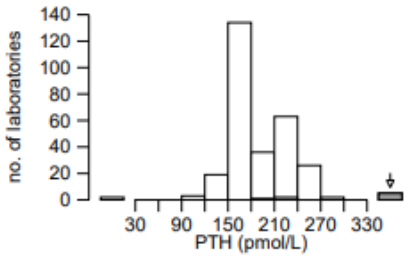
Specimen : P798

	n	Mean	GCV	Outl.
All methods	310	63.9	26.5	2
PTH Methods 1	285	64.4	25.5	1
Abbott Alinity	48	78.9	8.6	0
Beckman Access	32	66.8	9.9	1
Roche Elecsys	142	54.1	7.1	0
Siemens Atellica	38	81.8	7.4	0
PTH Methods 2	17	45.3	14.5	1
PTH STAT Methods	8	115.1	80.6	0
Future Diag STAT	5	159.8	18.2	0



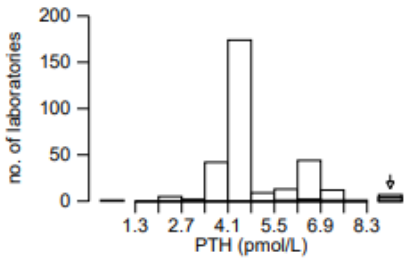
Specimen : P799

	n	Mean	GCV	Outl.
All methods	290	184.5	22.9	2
PTH Methods 1	266	185.8	21.8	2
Abbott Alinity	48	222.5	6.3	0
Beckman Access	32	197.5	6.7	1
Roche Elecsys	142	161.2	4.1	1
Siemens Atellica	21	254.6	5.5	0
PTH Methods 2	16	130.4	10.5	0
PTH STAT Methods	8	347.7	84.1	0
Future Diag STAT	5	467.6	35.6	0



Specimen : P800

	n	Mean	GCV	Outl.
All methods	310	4.7	21.4	4
PTH Methods 1	285	4.7	20.1	4
Abbott Alinity	48	6.5	6.6	0
Beckman Access	32	4.4	6.4	1
Roche Elecsys	142	4.3	4.1	3
Siemens Atellica	38	4.5	4.8	0
PTH Methods 2	17	3.4	24.2	0
PTH STAT Methods	8	8.5	45.1	0
Future Diag STAT	5	10.6	4.8	0



Comparison of NBCL, FD and Roche – % PTH drop & Precision

- Left over blood and plasma from patient samples collected during parathyroidectomies were analysed in duplicate on the Roche and NBCL platforms.
- Phase I (May-Nov 2021), Phase II (Jan-Mar 2022) - Improvements in clot detection and interference from heterophilic antibodies
- Phase III (Apr-Jul 2022): 13 patients, 72 samples measured in duplicate, interested in % CV ≤15%.
- Measurements in whole blood for NBCL, plasma for FD and Roche

	NBCL	FD	Roche
CV≤15% N, (%)	52, (72%)	71, (99%)	72, (100%)
CV>15% N (%)	20, (28%)	1, (1%)	0
Total No. of samples	72	72	72

- NBCL **not as precise** as FD and Roche when using **whole blood**
- However, the high % CVs would **not** have **changed** the **conclusion** of the surgery in **12 out 13** patients.
- For the only patient with contradicting results, measurement of **extra time point** would have helped to avoid further neck exploration

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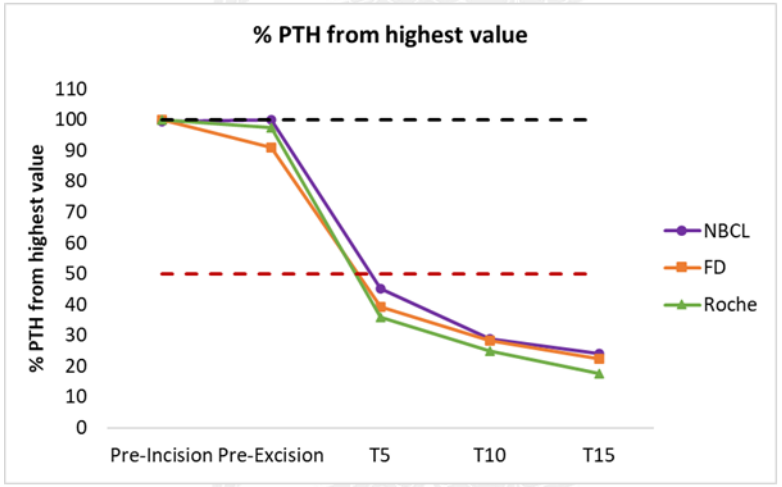
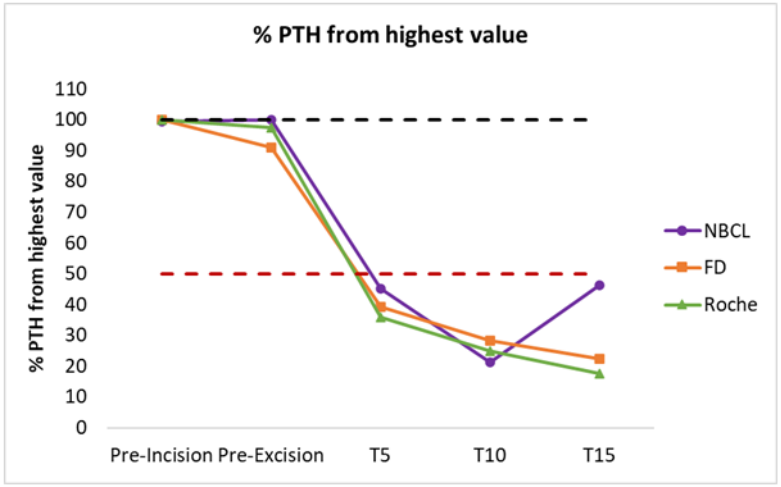
Comparison of NBCL, FD and Roche – % PTH drop & Precision

Highest value @T10
Lowest value @T15



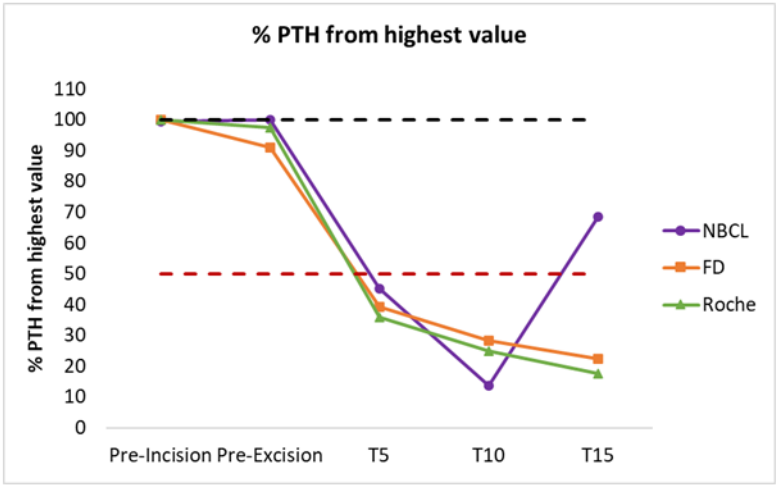
	PTH pmol/L			% PTH from highest value		
	NBCL	FD	Roche	NBCL	FD	Roche
Pre-incision	20.5	34.5	7.68	100	100	100
	19.6	36.1	7.59			
Pre-excision	21.5	30.8	7.44	100	91	98
	18.8	33.4	7.46			
T= 5 min	9.3	13.2	2.77	45	39	36
	8.89	14.6	2.70			
T= 10 min		9.3	1.87	29	28	25
	5.82	10.70	1.94			
T= 15 min		7.60	1.37	24	22	18
	4.83	8.1	1.33			

Mean values @
T10 and T15



	PTH pmol/L			% PTH from highest value		
	NBCL	FD	Roche	NBCL	FD	Roche
Pre-incision	20.5	34.5	7.68	100	100	100
	19.6	36.1	7.59			
Pre-excision	21.5	30.8	7.44	100	91	98
	18.8	33.4	7.46			
T= 5 min	9.3	13.2	2.77	45	39	36
	8.89	14.6	2.70			
T= 10 min	2.76	9.3	1.87	21	28	25
	5.82	10.70	1.94			
T= 15 min	13.8	7.60	1.37	46	22	18
	4.83	8.1	1.33			

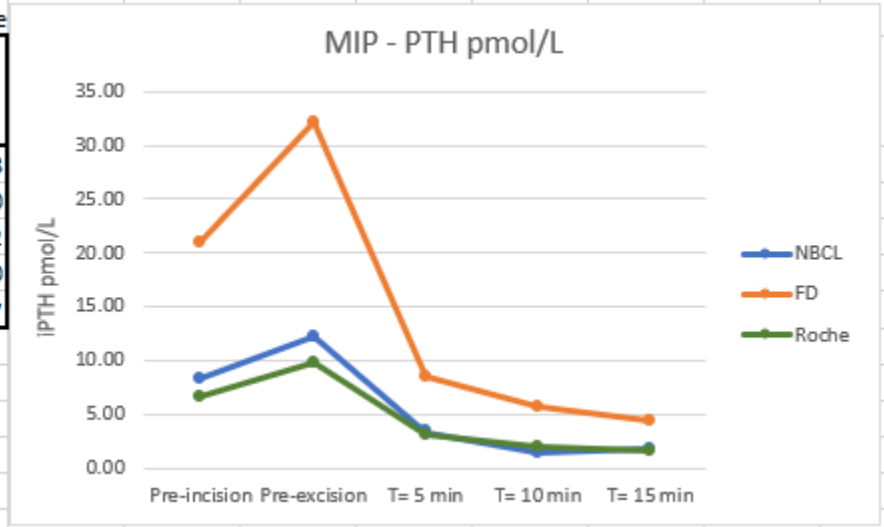
Lowest value @ T10
Highest value @T15

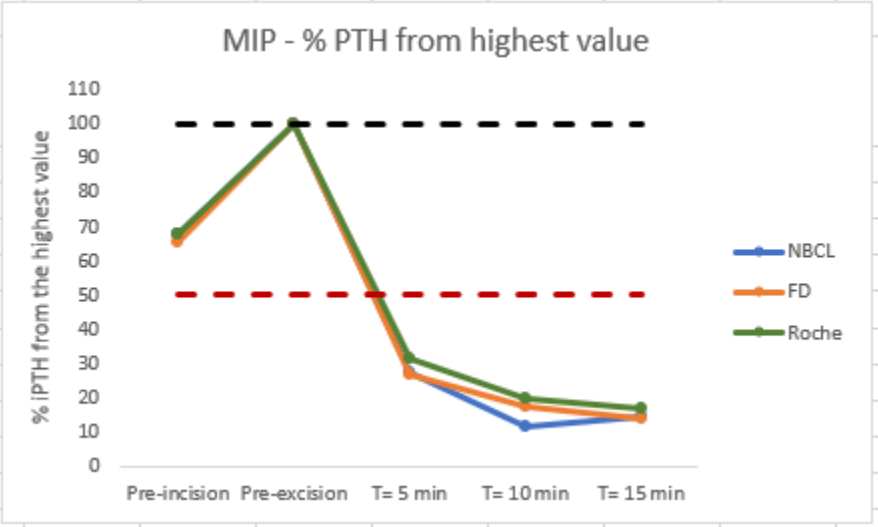


	PTH pmol/L			% PTH from highest value		
	NBCL	FD	Roche	NBCL	FD	Roche
Pre-incision	20.5	34.5	7.68	100	100	100
	19.6	36.1	7.59			
Pre-excision	21.5	30.8	7.44	100	91	98
	18.8	33.4	7.46			
T= 5 min	9.3	13.2	2.77	45	39	36
	8.89	14.6	2.70			
T= 10 min	2.76	9.3	1.87	14	28	25
		10.70	1.94			
T= 15 min	13.8	7.60	1.37	68	22	18
		8.1	1.33			

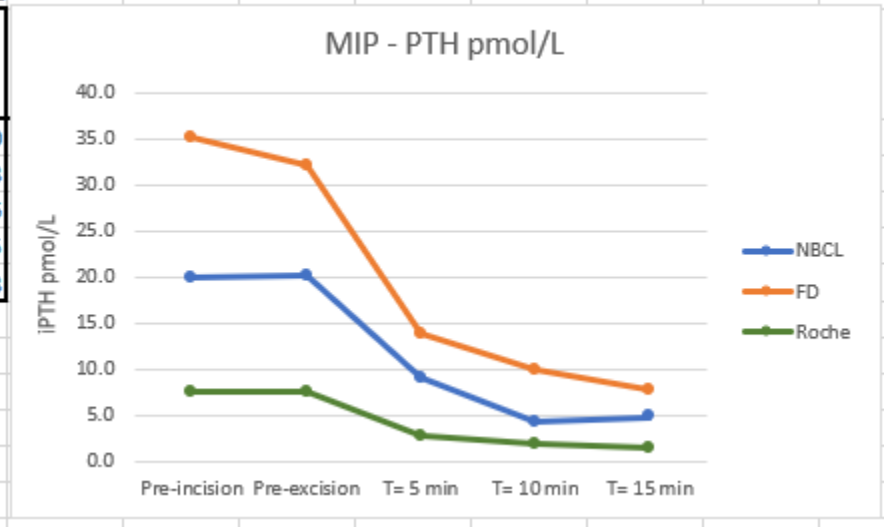
Comparison of NBCL, FD and Roche – % PTH drop

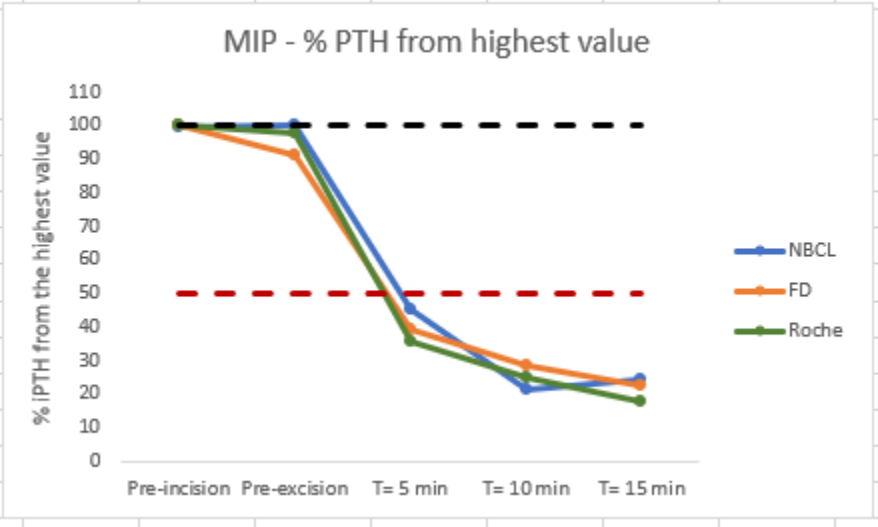
	PTH pmol/L			% PTH from highest value		
Patient 1	NBCL	FD	Roche	NBCL	FD	Roche
Pre-incision	8.31	21.05	6.60	68	66	68
Pre-excision	12.30	32.10	9.77	100	100	100
T= 5 min	3.40	8.55	3.09	28	27	32
T= 10 min	1.43	5.70	1.96	12	18	20
T= 15 min	1.80	4.40	1.65	15	14	17





	PTH pmol/L			% PTH from highest value		
Patient 2	NBCL	FD	Roche	NBCL	FD	Roche
Pre-incision	20.1	35.3	7.64	100	100	100
Pre-excision	20.2	32.1	7.45	100	91	98
T= 5 min	9.08	13.9	2.74	45	39	36
T= 10 min	4.29	10.00	1.91	21	28	25
T= 15 min	4.83	7.85	1.35	24	22	18





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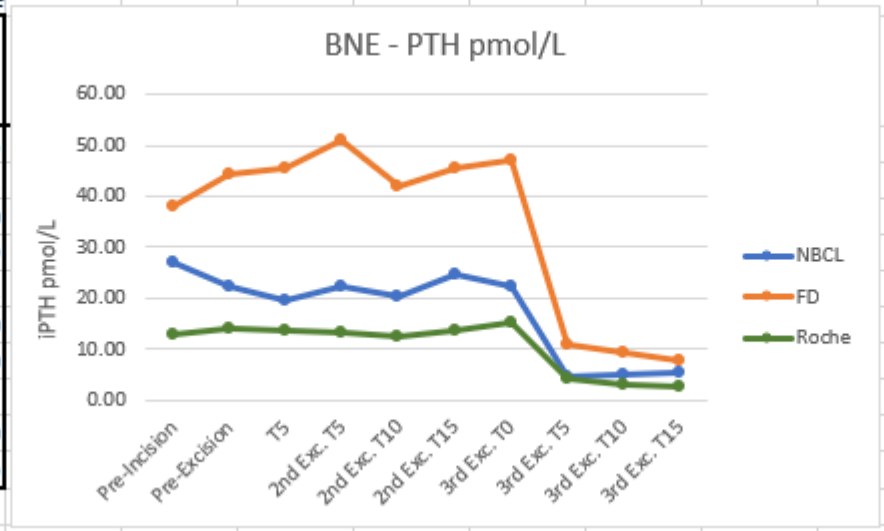
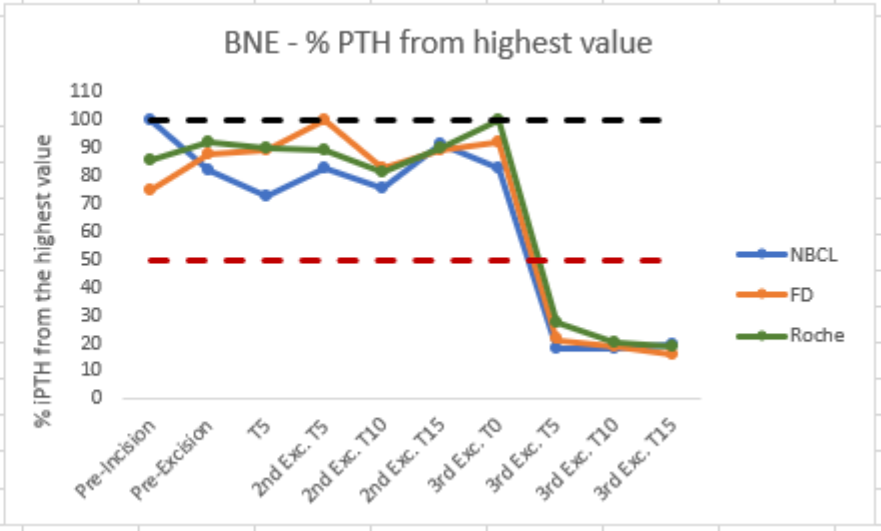
Comparison of NBCL, FD and Roche - % PTH drop

	PTH pmol/L			% PTH from highest value		
Patient 3	NBCL	FD	Roche	NBCL	FD	Roche
Pre-Incision	27.05	37.95	12.95	100	75	85
Pre-Excision	22.15	44.50	13.95	82	88	92
T5	19.65	45.40	13.65	73	89	90
2nd Exc. T5	22.45	50.85	13.5	83	100	89
2nd Exc. T10	20.45	42.00	12.35	76	83	82
2nd Exc. T15	24.80	45.35	13.6	92	89	90
3rd Exc. T0	22.30	46.95	15.15	82	92	100
3rd Exc. T5	4.85	10.80	4.15	18	21	27
3rd Exc. T10	4.89	9.50	3.02	18	19	20
3rd Exc. T15	5.34	8.00	2.845	20	16	19





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Conclusions & further work

	FD	NBCL
Comparability to laboratory method	Positively bias compared to Roche	Positively bias compared to Roche (company aims to align its method to Roche)
Precision	As precise as the Roche assay	NOT as precise as the Roche assay, but of limited impact
Cure Rate	Comparable to laboratory assay	Comparable to laboratory assay, but currently limited data
Grade of operator	Laboratory staff	Clinical staff
Pre-analytical considerations	Manual pipetting	Avoid samples collected directly from the drip arm
Surgery planning	Not flexible – 2x per month	Flexible
Cost per patient	£400 (reagents plus staff time)	£440 - Expectation to improve the overall pathway by being able to plan operations more flexibly.

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NBCL

- Finalise the product, define the LLOQ, minimise variability among cartridge lot numbers
- Apply for FDA and IVDR approval in 2025

Thank you!
Any Questions?

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