28th November 2025

Royal College of Pathologists

NHS
Black Country Pathology Services

National Audit on Testosterone

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Testosterone

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- An androgenic steroid hormone produced in the testis
- Primary sex hormone and androgen in males, regulating sex differentiation and development and maintenance of secondary sexual characteristics
- Produced in smaller amounts in females in the ovaries and adrenal glands
- Main indications for measurement in adults:
  - **Identification of male hypogonadism**
  - **Identification of female hyperandrogenism**
  - Monitoring of testosterone therapy
  - Monitoring of gender affirming hormone therapy

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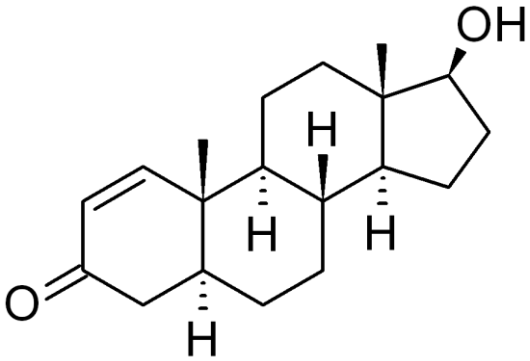

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Male hypogonadism

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- Clinical syndrome characterised by impaired testicular function
  - Deficient testosterone production and impaired spermatogenesis
- Diagnosed through clinical signs & symptoms with corroborative biochemical evidence of testosterone deficiency
- Clinical features include erectile dysfunction, low sexual desire, low sperm count, low haemoglobin/haematocrit, gynaecomastia, decreased bone density
- May be primary (testicular dysfunction) or secondary (pituitary / hypothalamic dysfunction) or functional…

Female hyperandrogenism

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- Clinical or biochemical evidence of increased androgenic steroids *e.g.,* testosterone
  - Androgen production may be increased in the ovaries and/or adrenals
- Clinical features of androgen excess include hirsutism, acne, menstrual cycle irregularities, infertility
  - If prolonged/severe may result in virilisation – clitoromegaly, deepening of voice, male-pattern baldness, erthyrocytosis
- Usually associated with polycystic ovary syndrome (PCOS)
- Other causes include non-classical congenital adrenal hyperplasia (CAH), adrenal or ovarian tumours, ovarian hyperthecosis, Cushing Syndrome, acromegaly, severe insulin resistance

Aim of national audit

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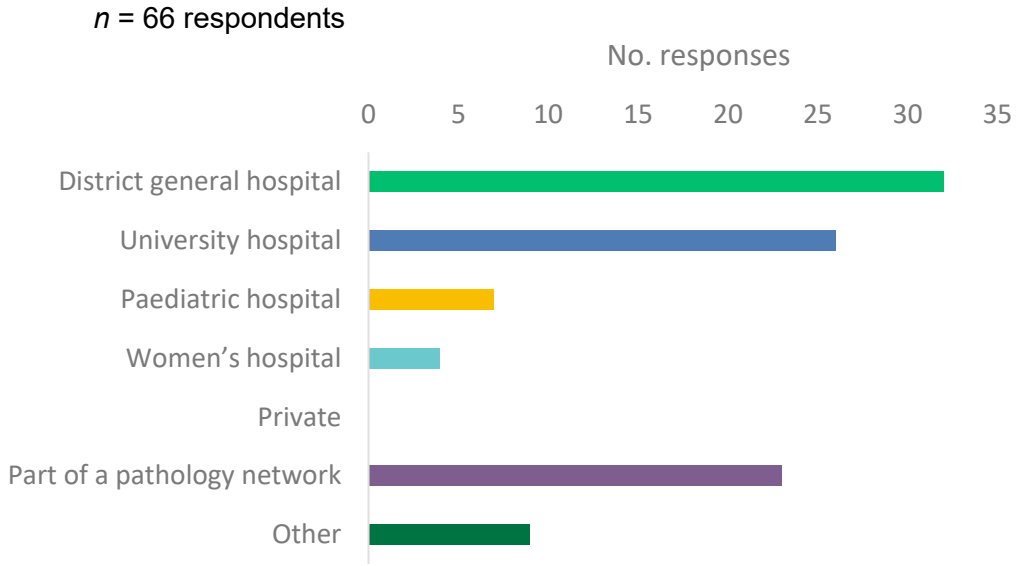
To capture current practice around requesting, measuring and reporting of testosterone, and other related biochemical parameters, in the investigation of male hypogonadism and female hyperandrogenism in adults.

Audit covered:

- male and female testosterone
- sex hormone binding globulin (SHBG)
- free androgen index (FAI)
- calculated free testosterone (FT)
- 17-hydroxyprogesterone (17OHP)
- D4-androstenedione (D4A)
- dehydroepiandrosterone sulphate (DHEAS)

Responses

- Survey Monkey questionnaire (24 questions) distributed to all Association of Laboratory Medicine members
- 66 sites responded
- Representation from England, Northern Ireland, Scotland and Wales



Locally agreed clinical pathways

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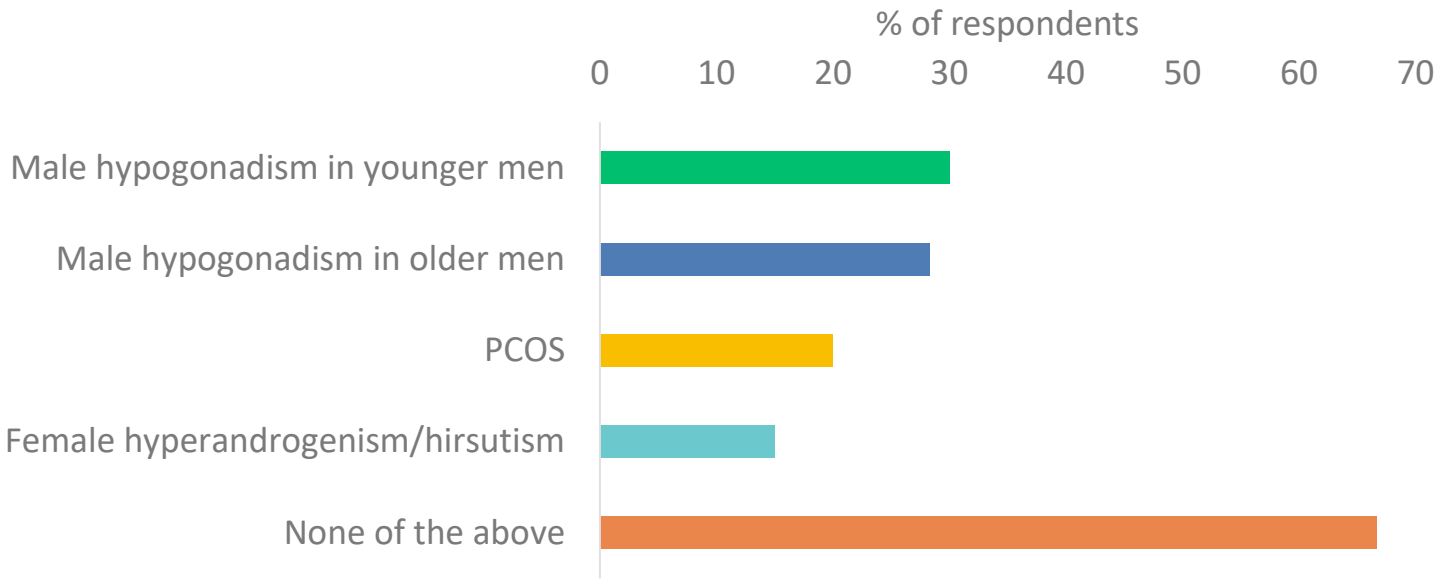
n=60 respondents



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Aspects of male testosterone

[SfE 2022] Society for Endocrinology Guidelines for Testosterone Replacement Therapy in Male Hypogonadism. Clin Endocrinol. 2022; 96(2): 200-219

[BSSM 2023] The British Society for Sexual Medicine Guidelines on Male Adult Testosterone Deficiency, with Statements for UK Practice. World J Mens Health 2023; 41:e33

Pre-analytical requirements for testosterone

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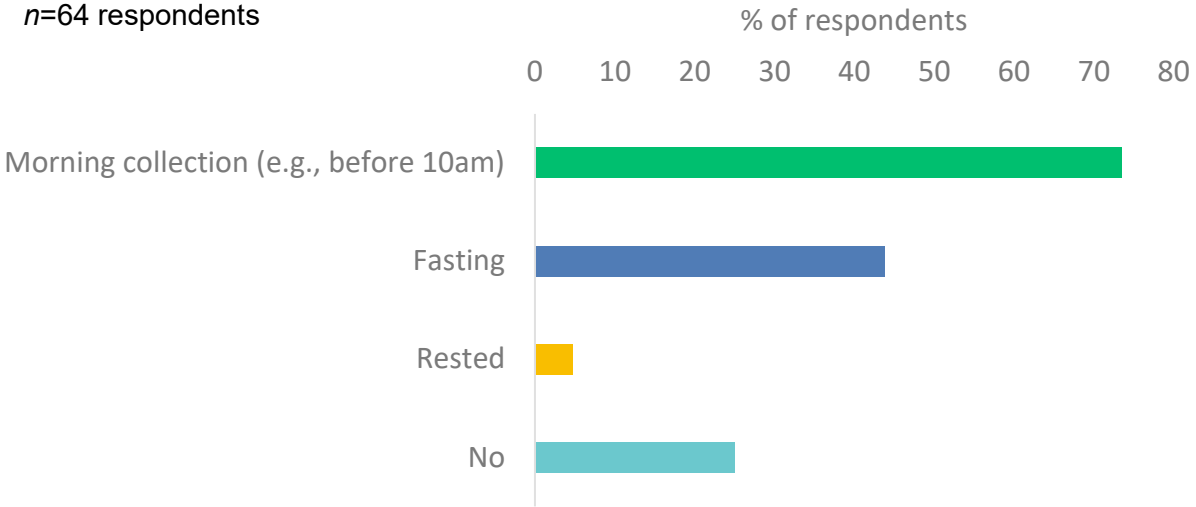
n=64 respondents



BSSM 2023:

- Measure testosterone in morning (before 11am), "ideally fasting"

SfE 2022:

- Collect testosterone under standardised conditions (8-10am, fasted and well rested)

Most common modes of communication to users:

- Report comment when suggesting repeat testosterone (66.7%)
- User handbook/ test database for users (47.4%)
- Electronic requesting system (21.1%)

Primary method for measuring testosterone

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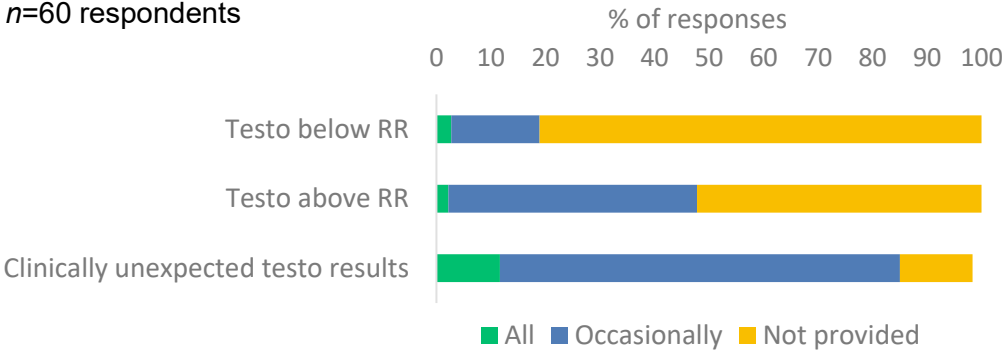
- 98.5% (64/65) sites use immunoassay
- 41% use age-related reference ranges
  - Most (*n*=31) are < and > 50y
- Reference range sources;
  - 75% manufacturer's kit insert, 8% in-house study, 8% literature

Confirmation of testosterone by LC-MS/MS

n=60 respondents



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BSSM 2023:

- Measure testosterone using reliable method
- Improvements in standardisation of testosterone assays required
- >80% of men maintain normal testosterone levels into old-age (with increasing SHBG) but increased incidence of testosterone deficiency due to comorbidities

SfE 2022:

- Some immunoassays out-perform LC-MS/MS in male range
- Age-related decline in serum testosterone (particularly FT), but often due to accumulating comorbidities

Free testosterone

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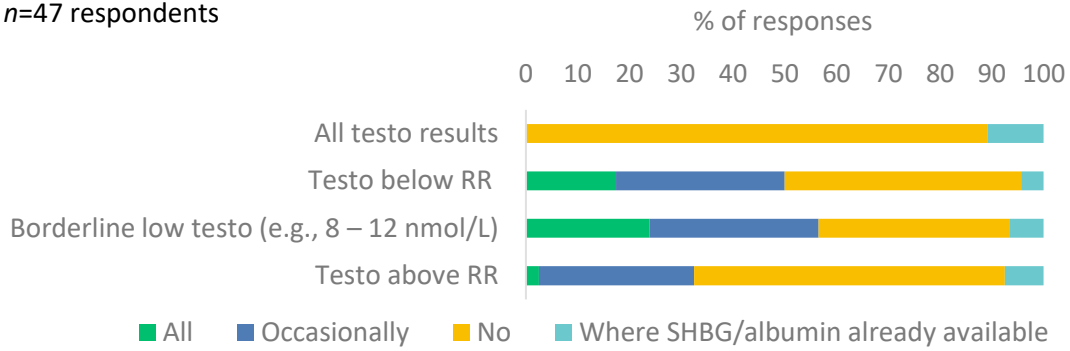
- 94.6% (*n*=35/37) of those reporting FT use the Vermeulen equation and 2 labs use a modified equation (testosterone and SHBG only)
- 11 sites report FAI in males

When is free testosterone reported?

n=47 respondents



- FT available as direct request:
  - 1° care: 33.8%, 2° care: 65.2%

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BSSM 2023:

- Calculate FT when testo 8-12 nmol/L or where suspected/ known abnormal SHBG
- Calculate using Vermeulen equation (FAI not recommended)
- Testosterone tx indicated in symptomatic men with confirmed FT <0.225 nmol/L

SfE 2022:

- Calculate FT when SHBG abnormal or testosterone borderline low with clinical features of deficiency
- Accuracy limited by testosterone and SHBG assay performance
- Ref ranges & decision limits should be specific for assays used

Defined cut-off for testosterone to suggest hypogonadism

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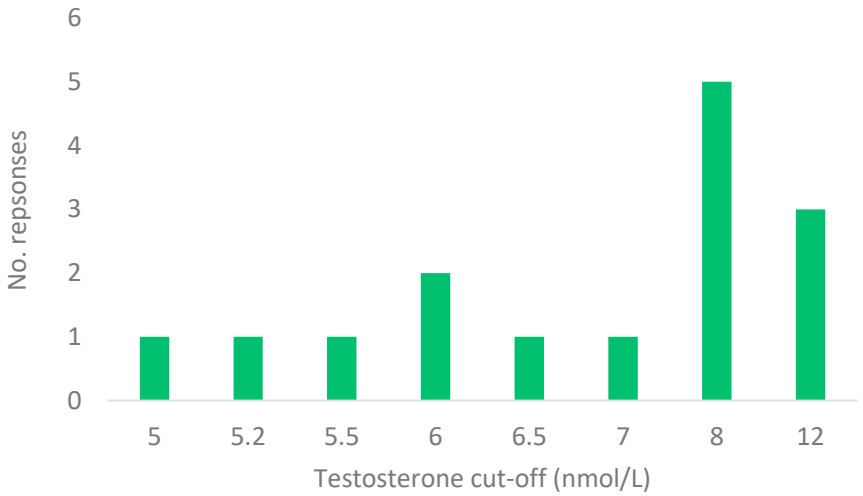
- 71.9% (*n*=46/64) labs rely on the lower limit of the reference range to suggest hypogonadism
- 28.1% (*n*=18/64) labs have a defined cut-off



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BSSM 2023:

- Base therapy decisions on evidence-based action levels in symptomatic men rather than lab reference ranges
- Confirmed morning testosterone <12 nmol/L with symptoms usually requires testosterone tx

SfE 2022:

- <8 nmol/L - high suspicion of hypogonadism (assuming normal SHBG)
- Important to evaluate in context of clinical findings

Recognition of a borderline low testosterone concentration range in reporting

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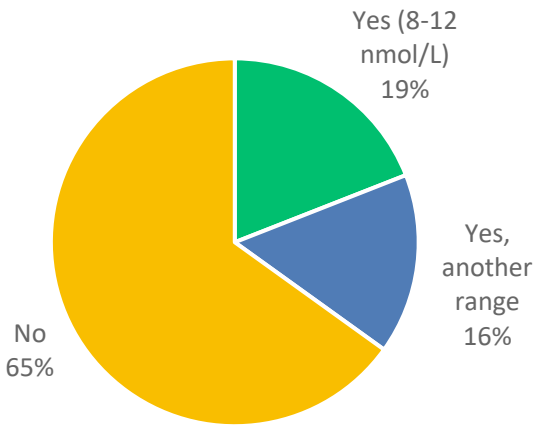
n=63 respondents



Other borderline low testosterone ranges (nmol/L)
4-LLN (depends on age)
5.5-9.9
6-10
6-12 (for calculating FAI)
6.5-8.8 (<50y), 4.6-8.6 (>50y)
7-11
7-14
8-11 (*n*=2)
LLN (8.6 or 6.7)-12

BSSM 2023:

- 8-12 nmol/L for calculating free testosterone
- 8-14 nmol/L may require trial of testosterone therapy, depending on symptoms

SfE 2022:

- Range of 8-12 nmol/L falls between high and low suspicion of testosterone deficiency
- Recommend calculation of FT in this group

Reflex tests in response to male testosterone results

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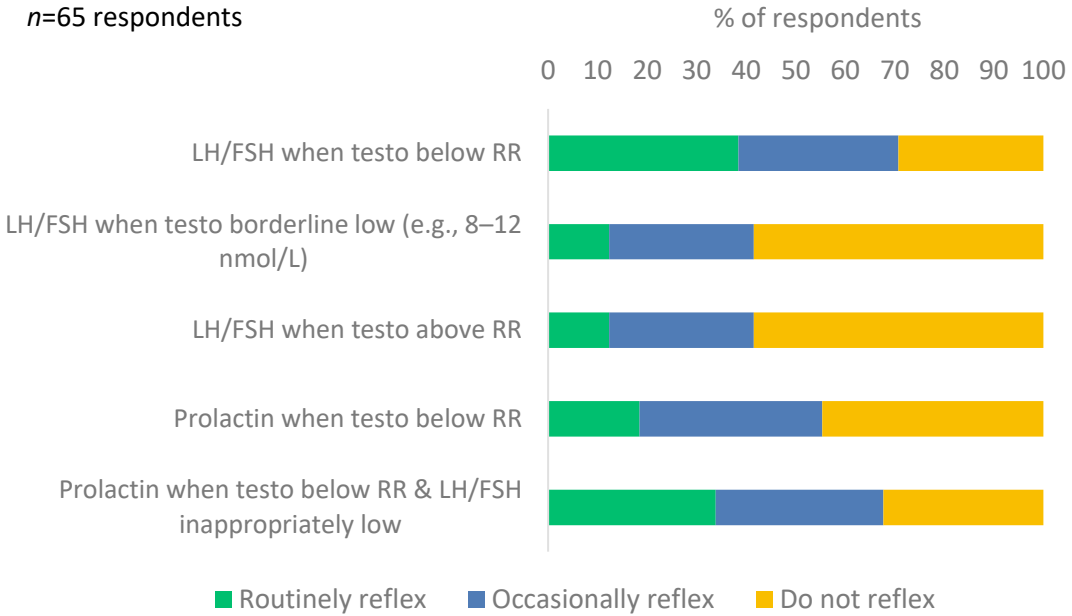

N.B. Some add comment to advise tests on subsequent sample

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BSSM 2023:

- Measure LH/FSH when testosterone <12 nmol/L to differentiate 1° from 2° hypogonadism and to identify testicular failure when testosterone is "low-normal"
- Measure prolactin when LH and FSH are low, particularly if testosterone <5.2 nmol/L

SfE 2022:

- Measure LH to differentiate 1° from 2° hypogonadism
- Pituitary biochemical profiling/imaging recommended as 2nd line investigations in 2° hypogonadism

When adult male testosterone is below the reference range, what information does your laboratory's comment convey?

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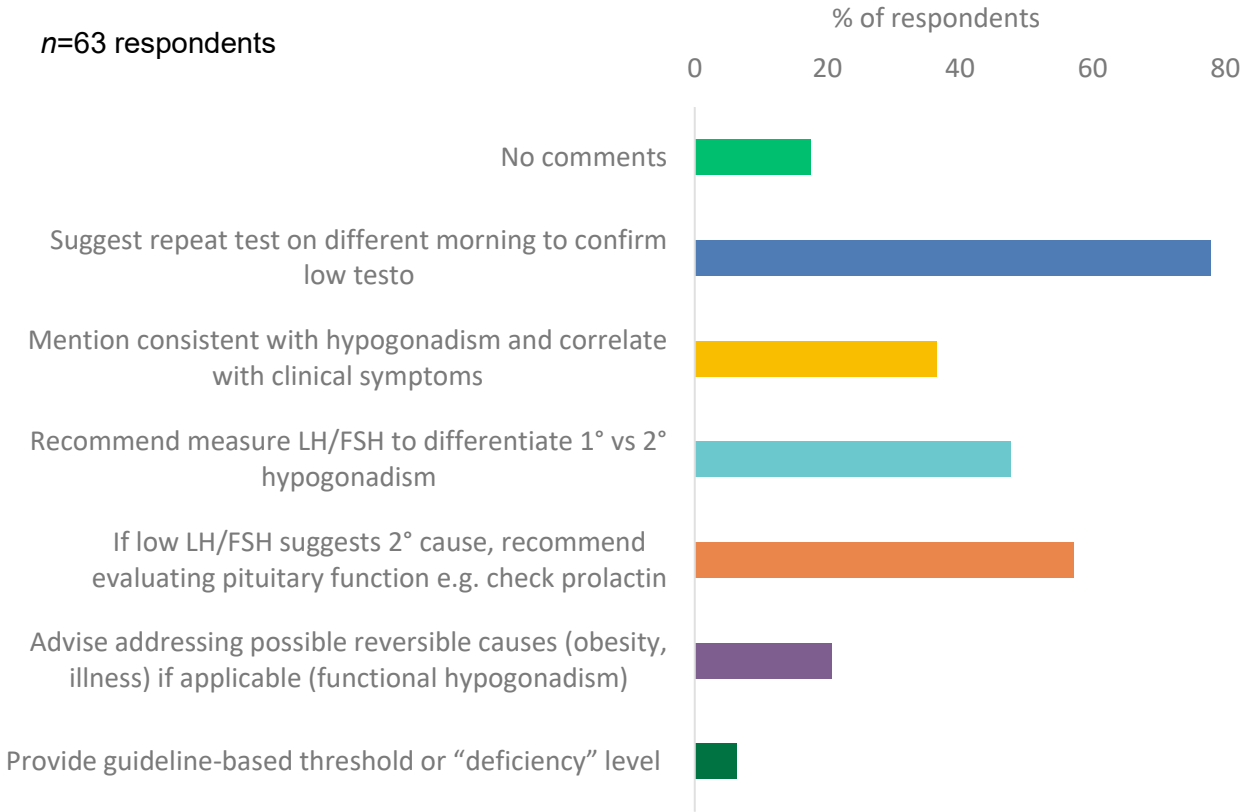

BSSM 2023:

- Repeat testosterone on ≥2 separate mornings
- With symptoms, confirmed testosterone <12 nmol/L or FT <0.225 nmol/L indication for testosterone tx
- Address potentially reversible causes in conjunction with testosterone tx if symptomatic/ testosterone <12 nmol/L

SfE 2022:

- Interpret results in context of clinical symptoms
- Advise addressing reversible causes rather than testosterone tx in "functional hypogonadism"

For borderline low testosterone results (e.g., 8–12 nmol/L) in adult males, what information does your laboratory's comment convey?

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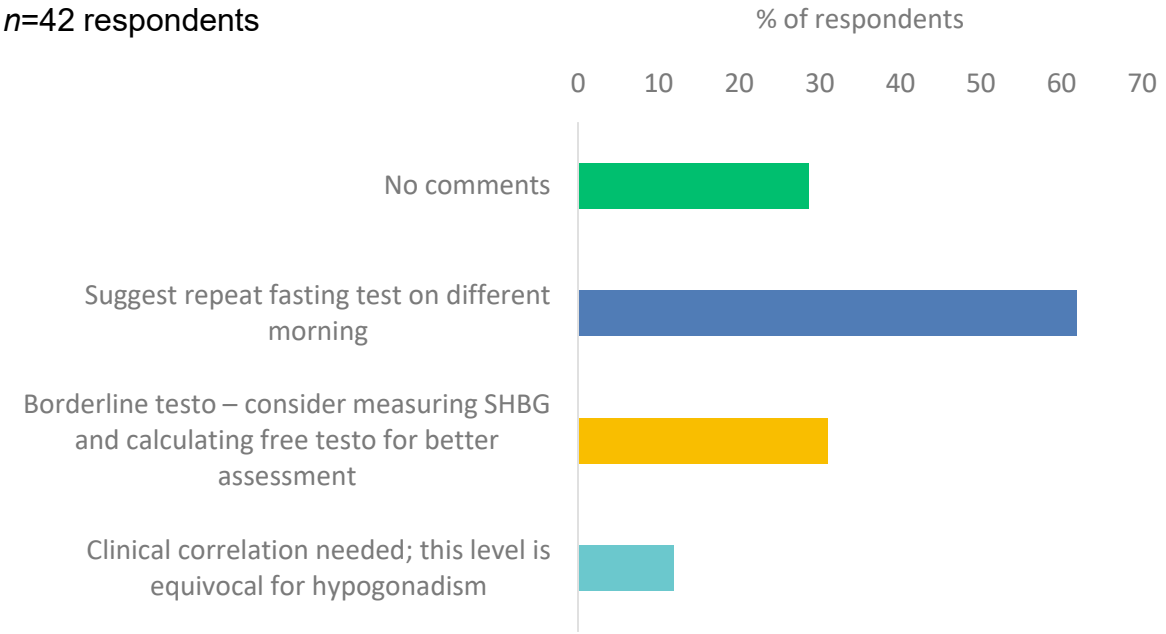
n=42 respondents



BSSM 2023:

- Repeat testosterone on ≥2 separate mornings
- Measure SHBG and calculate free testosterone
- Testosterone 8-14 nmol/L may require trial of testosterone therapy, depending on symptoms

SfE 2022:

- Calculate free testosterone

Summary

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- Only a third of sites have a locally agreed protocol for male hypogonadism
- Mixed picture around use of age-related testosterone reference ranges and unclear whether this is best practice or not
- Some sites report FAI in males rather than calculated FT and most labs don't routinely report FT in borderline low testosterone results
- Majority of labs rely on lower limit of testosterone reference range to suggest hypogonadism. Where a defined cut-off is used, the values span a wide concentration range
- Most labs don't recognise a borderline low testosterone concentration range
- Laboratory comments frequently advise repeat on another day and suggest measurement of FSH/LH. Comments less frequently cover advising on whether result is consistent with hypogonadism, to correlate with clinical picture and to address reversible causes

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Aspects of female testosterone

[SfE 2025] Society for Endocrinology Clinical Practice Guideline for the Evaluation of Androgen Excess in Women. Clin Endocrinol. 2025; 103(4): 540-566

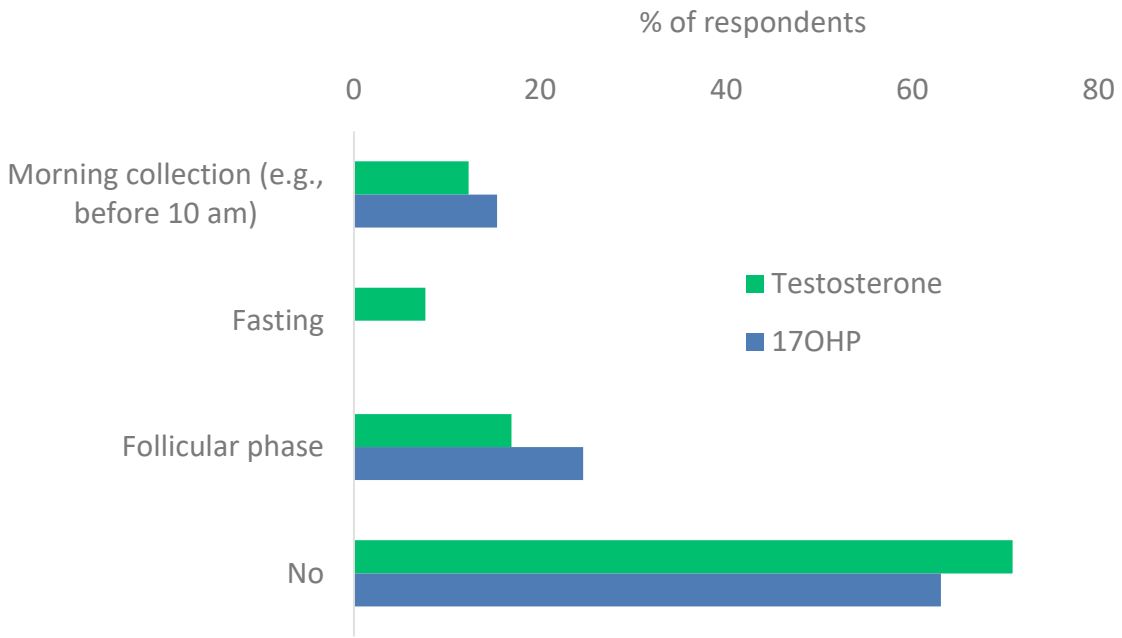
[ES 2018] Endocrine Society Clinical Practice Guideline: Evaluation and treatment of hirsutism in pre-menopausal women JCEM 2018; 103(4): 1233-1257

[PCOS 2023] International Evidence-Based Guideline for the Assessment and Management of Polycystic Ovary Syndrome 2023

Pre-analytical requirements

Black Country Pathology Services

n=65 respondents



SfE 2025:

- Collect samples for investigation of androgen excess (including testosterone & 17OHP/D4A/DHEAS) between 8-10 am, following overnight fast, in early follicular phase where possible
- Most evidence-based reference ranges derived from fasted, early morning follicular samples

ES 2018:

- Screen hyperandrogenaemic women for non-classical CAH using early morning 17OHP in follicular phase

Primary testosterone method

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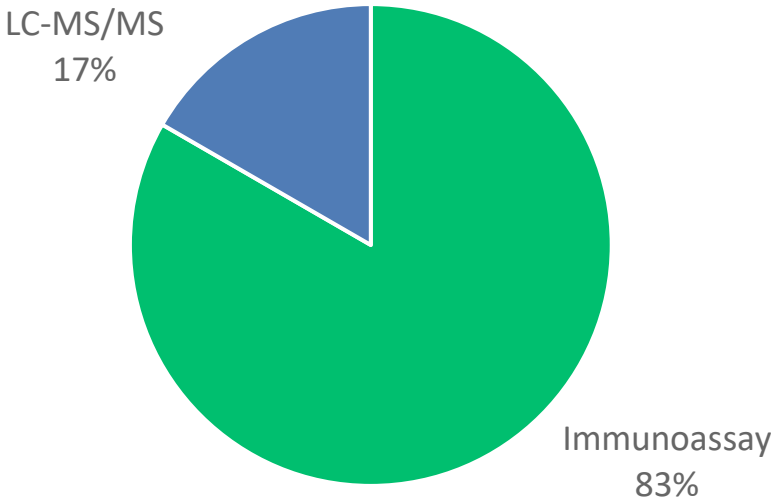
n=66 respondents



SfE 2025:

- Use validated, highly accurate LC-MS/MS testosterone measurement where possible
- Most immunoassay methods exhibit bias (positive or negative) *vs*. LC-MS/MS in female testosterone range
- Immunoassay methods exhibit cross reactivity at low circulating androgen concentrations

ES 2018:

- Measure testosterone using a reliable, speciality assay

PCOS 2023:

- Should use highly accurate LC-MS/MS assays for measuring total testosterone and for D4A/DHEAS where required

Confirmatory LC-MS/MS

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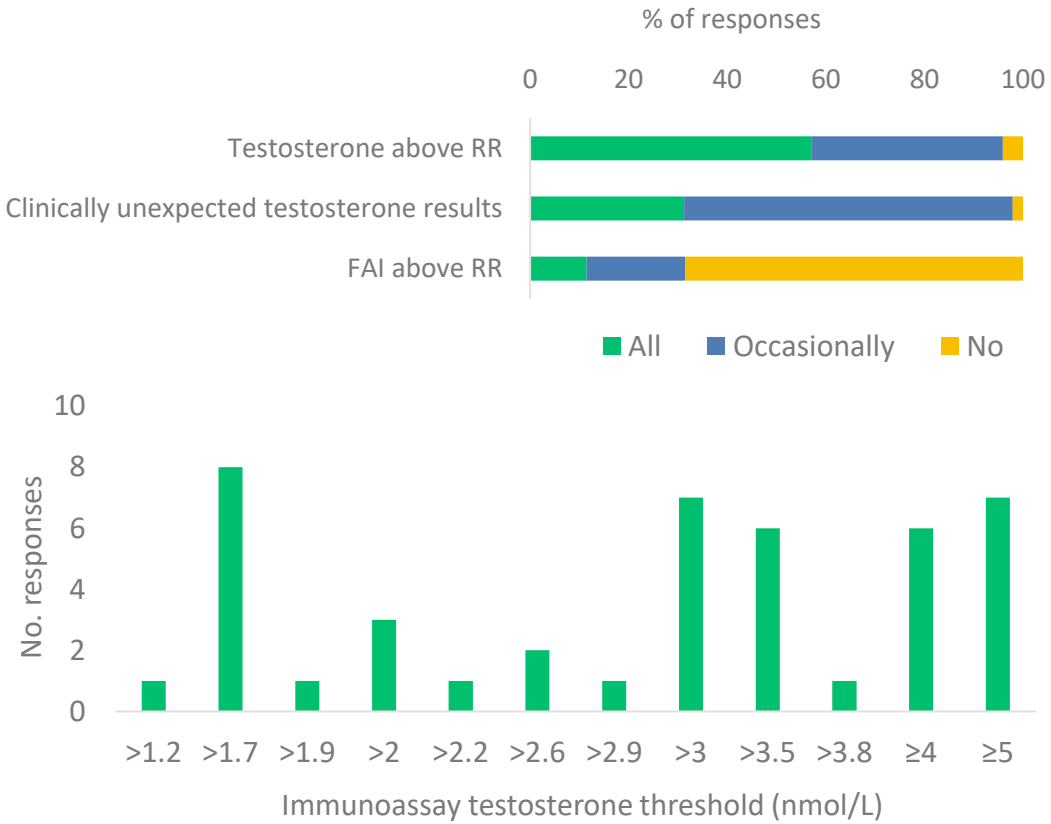
n=49 respondents



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- Some labs indicated “at DB discretion only”, many stated threshold used with DB review
- Indications for not confirming results included “on testosterone”, “transgender”, “previous LC-MS/MS result” and “pregnancy”
- FAI-based thresholds (*n*=8) ranged from >4.5 to >12%

SfE 2025:

- Testosterone results that do not align with the clinical picture or >5 nmol/L warrant validation with LC-MS/MS

Age-related reference ranges

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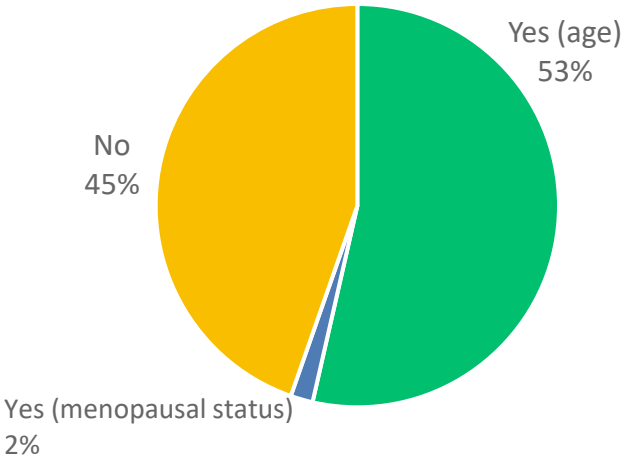
Immunoassay

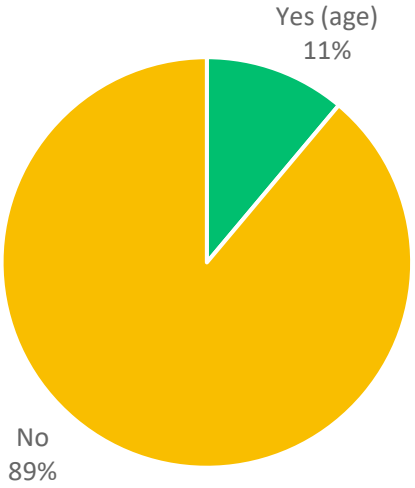
n=56 respondents



- 71% from manufacturer's kit insert
- 8% from in-house study
- 3% from literature
- Age-related ranges < and >49/50y

LC-MS/MS

n=27 respondents



- 43% from in-house study
- 24% from literature

SfE 2025:

- Interpret testosterone *vs*. evidence-based reference ranges
- Age and/or menopausal status specific

PCOS 2023:

- Current reference ranges not reliable for diagnosing biochemical hyperandrogenism
- Labs should establish their own reference ranges in well characterised, healthy control population

Measurement/reflex of SHBG/FAI

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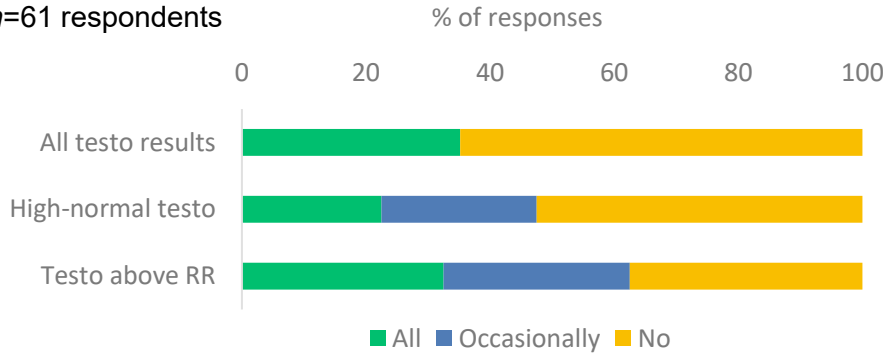
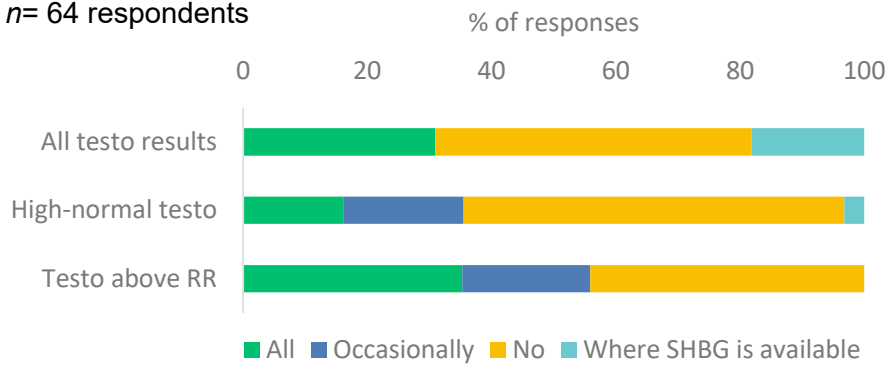
SHBG (90% have available as a direct request)

n=61 respondents



FAI (66% have available as a direct request)

n= 64 respondents



SfE 2025:

- Recommend SHBG and an index of FT in all women with clinical features/ suspicion of androgen excess
- Calculated FT more robust correlation with measured FT *vs.* FAI

PCOS 2023:

- Assess biochemical hyperandrogenism using total and free testosterone (calculated or measured)

ES 2018:

- Where total testosterone is normal & clinical evidence of hyperandrogenism, measure early morning serum total testosterone and FT (calculated or equilibrium dialysis)

Measurement/reflex of other serum androgens

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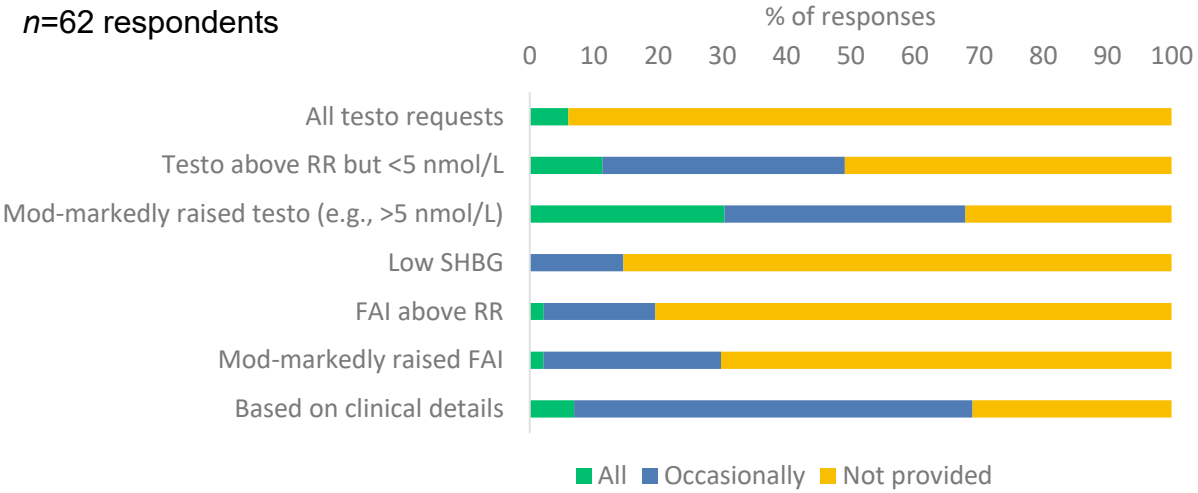
n=62 respondents



- 13 sites: no testosterone threshold for reflexing other serum androgens, use clinical judgement
- Testosterone thresholds (*n*=21) ranged from >1.7 to 5 nmol/L (*n*=21), with >4.0 nmol/L (*n*=6) and >5.0 nmol/L (*n*=4) being most common

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SfE 2025:

- 17OHP/D4A/DHEAS to be requested in women presenting with clinical features/suspicion of androgen excess

ES 2018:

- Measure 17OHP in hyper-androgenaemic women to screen for non-classical CAH
- Measure 17OHP in hirsute patients with a high risk of CAH even if testosterone/FT normal

PCOS 2023:

- If testosterone not elevated, consider measuring D4A and DHEAS

If testosterone is raised, what information does your laboratory's comment convey?

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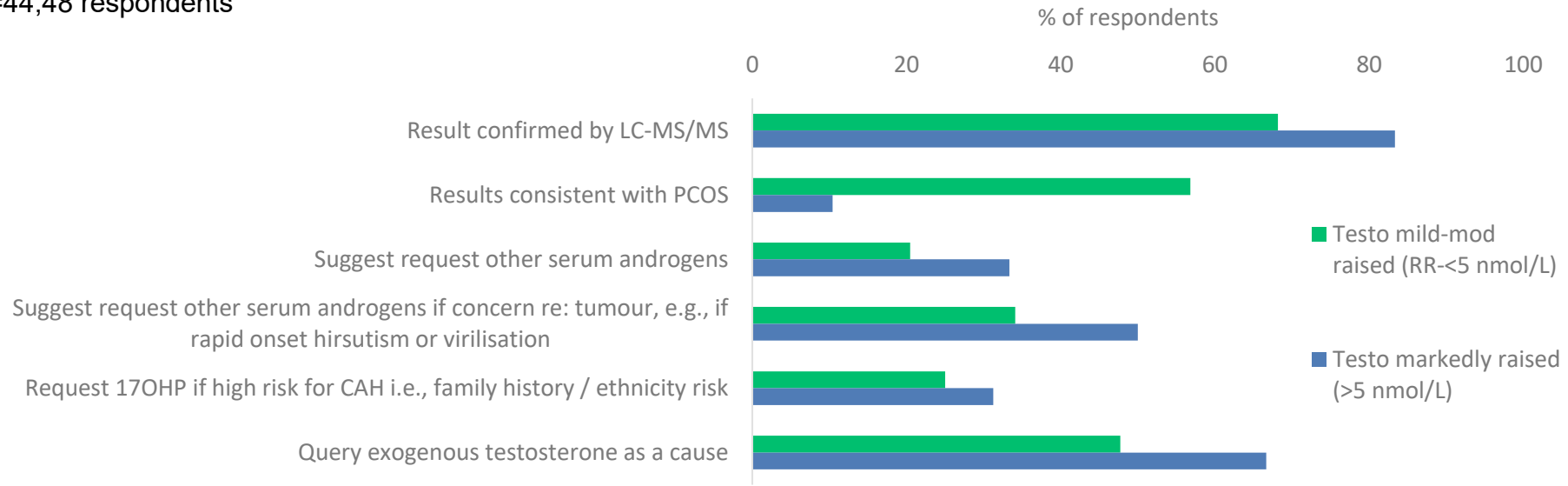
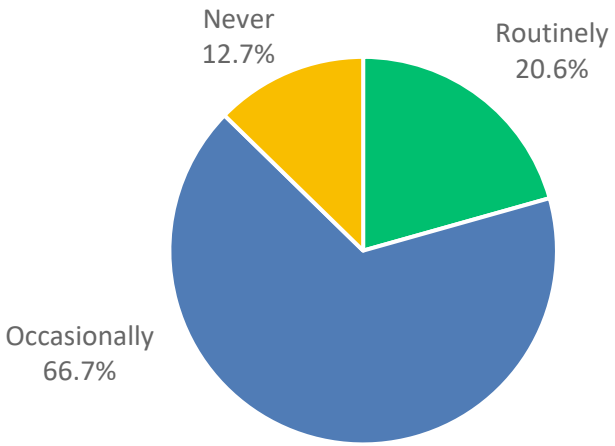
n=44,48 respondents



PCOS 2023: Consider causes other than PCOS if androgen levels are markedly increased

- Some androgen secreting neoplasms associated with mild/moderate increases in androgens. Clinical history of time of onset +/-rapid progression of symptoms key in assessment

Urgent communication of female testosterone results



- 28/55 (50.9%) had a specific threshold above which they would consider phoning results, others on a case-by-case basis only
- Most common threshold was >5 nmol/L (*n*=18) with a range of >3 to 10 nmol/L

S4E 2025:

- Testosterone >5 nmol/L classed as “severe androgen excess” warrants urgent follow-up with ovarian and/or adrenal imaging
- Older age and rapid onset / progression are predictive factors for non-PCOS pathology

ES 2018:

- Urgent referral to Endocrinology warranted if clinical features suggestive of androgen secreting tumour or testosterone is >6-7 nmol/L
- Refer for further investigation if testosterone >4 nmol/L or 17OHP raised

Conclusions

Black Country Pathology Services

- Less than 20% of sites have locally agreed protocols for PCOS and/or female hyperandrogenism
- Few labs communicate any pre-analytical requirements for female testosterone/androgens to users
- LC-MS/MS not commonly used as primary method for female testosterone, although recommended in guidance. Confirmation of raised and clinically unexpected immunoassay results often undertaken
- In many labs, FAI not routinely reported in all women with clinical features/suspicion of androgen excess
- Majority of sites only reflex serum androgens in specific clinical circumstances or if testosterone is markedly raised. New guidelines recommend measuring serum androgens first-line in women with clinical features/suspicion of androgen excess
- Interpretative comments reflect the different interpretation of mild-moderate raised androgens and severely raised androgens
- Most labs urgently communicate raised testosterone results when clinical details are of concern but only half have a guidance threshold for testosterone

Acknowledgements

Dr Tejas Kalaria, Consultant Chemical Pathologist BCPS
Dr Anna Sanders, Consultant Clinical Scientist, BCPS

Dr Wassif and Louise Ward
Association Laboratory Medicine National Audit Committee

Everyone who participated in the survey

Thanks for listening