**PLASMA METANEPHRINES**

**INSTRUCTIONS FOR USERS AND REQUESTING CLINICIANS**

1. **Sample Requirements**
   1. EDTA whole blood samples are preferred (although heparin samples can be used).
   2. Minimum sample volume: 1 mL EDTA whole blood
   3. Minimum assay volume: 200 µL plasma
2. **Collection Procedures and Sample Stability**

2.1 Whole blood samples should be transported to the department on ice and arrive within 2 hours of sampling. Samples not transported on ice MUST arrive at the department within 30 minutes of collection to be viable for analysis. Any blood samples not fulfilling these collection criteria will not be assayed.

2.2 Plasma samples sent through the post should arrive frozen, packed with dry ice or ice packs to maintain a temperature below 8°C. Plasma samples that arrive thawed but cold (<8°C) and within 3 days of posting are still acceptable. Plasma samples that arrive thawed and at room temperature in the postage box are NOT suitable for analysis.

2.4 Frozen samples are stable for at least 6 months. Other storage conditions are not suitable.

1. **Patient Preparation and Posture During Sampling**

3.1 PATIENT POSTURE DURING SAMPLING

Routine practice in Newcastle clinics (and many other UK centres) is to collect samples from seated patients for plasma metanephrines (although some endocrinology patients undergo supine sampling when visiting the day case unit e.g. patients with susceptibility syndromes). The tabulated reference ranges used in our reports are based on a seated population, but we also include lower supine reference ranges in the comments. There are published articles and guidelines (1,2) suggesting that supine sampling (after 30 minutes supine rest) is preferable, given the apparent improvement in diagnostic performance. It has also been suggested that if seated sampling is used, lower reference ranges based on a supine reference population should be applied in order to maximise diagnostic sensitivity (2). However, we do not recommend applying supine reference ranges when interpreting results from seated patients due to the large increase in the number of false positive results that would be observed (3).

Although local data suggests that a seated sampling approach achieves high diagnostic sensitivity (3), supine sampling is considered the ideal sampling protocol, given the improved diagnostic performance that may be achieved (2). Supine sampling may be more important in patients who potentially harbour smaller, pre-symptomatic tumours (e.g. patients undergoing surveillance due to a predisposition syndrome or previous PPGL). Where supine sampling is used, patients should be recumbent for 30 minutes before sampling (1,2). It is important that appropriate reference ranges derived from a supine reference population are used to interpret results from samples taken in the supine posture (see section 4).

3.2 DIET/FASTING STATUS

Fasting status and the impact of dietary catecholamines have minimal impact on concentrations of plasma free normetanephrine and metanephrine. However, dietary catecholamine intake (and potentially intake of some non-catecholamine rich foods) can significantly increase plasma 3-methoxytyramine concentrations. Overnight fasting and avoidance of catecholamine-rich foods (e.g. bananas, plums, pineapples, walnuts, tomatoes, avocados, aubergines, alcoholic drinks, vinegar) is advised if measurement of 3-methoxytyramine is likely to be important as a marker of dopamine secretion (4). NB: urine de-conjugated (total) metanephrines are more likely to be influenced by diet.

3.3 DRUGS/MEDICATIONS

Many drugs and medications may interfere pharmacodynamically with measurement of plasma metanephrines, potentially causing false positive results. The LC-MS/MS method for plasma free metanephrines is less susceptible to analytical interference than other methods. However, there is evidence that the vasopressor midodrine may interfere in some LC-MS/MS assays (9). Pharmacodynamic interference involves the effects of drugs on secretion, metabolism and excretion of catecholamines or metabolites.

The most troublesome causes of false positive results are from medications that block neuronal reuptake of norepinephrine, i.e. tricyclic antidepressants, ‘selective’ serotonin reuptake inhibitors and serotonin/norepinephrine reuptake inhibitors. Other potential causes of false positive results include anti-hypertensive drugs (e.g. α- and β-adrenergic receptor blockers and calcium channel blockers), monoamine oxidase inhibitors, Dopa-related drugs, and various sympathomimetic and stimulant drugs (see Table 1).

Ideally patients should discontinue all medications that may affect plasma and urinary catecholamines or metanephrines concentrations prior to sampling. In practice, it is not always possible to discontinue medication before testing and it may be more practical to carry out repeat testing only when initial tests are elevated.

|  |  |
| --- | --- |
| **Drug class** | **Examples** |
| Tricyclic antidepressants | Amitriptyline, clomipramine, dosulepin |
| Selective serotonin reuptake inhibitors | Citalopram, fluoxetine, sertraline |
| Serotonin/noradrenaline reuptake inhibitors | Venlafaxine, duloxetine |
| α-adrenergic receptor blockers | Phenoxybenzamine, doxazosin, indoramin |
| β- adrenergic receptor blockers | Atenolol, labetalol, propanolol |
| Calcium-channel blockers | Amlodipine, diltiazem, nifedipine |
| Monoamine-oxidase inhibitors | Isocarboxazid, phenelzine, moclobamide |
| Dopa-related | Levo(L)-Dopa, methyldopa |
| Stimulant / Sympathomimetic drugs | Ephedrine, amphetamine, cocaine, nicotine, caffeine |

**Table 1**. Medications with potential to cause false positive plasma metanephrines results.

1. **Reference Ranges and Diagnostic Cut-Offs**

4.1 SEATED REFERENCE RANGES

The reference ranges included in our reports are based on a seated reference population:

*Plasma normetanephrine:* <1180 pmol/L

*Plasma metanephrine:* <510 pmol/L

*Plasma 3-methoxytyramine:* <180 pmol/L

A summary of our interpretive guidance is as follows:

|  |  |  |
| --- | --- | --- |
|  | **Metanephrines Ranges** | **Interpretation** |
| **Within Reference Range** | NMET: <1180  MET: <510 | These results do not suggest the presence of a phaeochromocytoma |
| **Borderline**  **Up to 2x ULRR** | NMET: 1181-2360  MET: 511-1020 | Borderline elevation in metanephrines.  Phaeochromocytoma/paraganglioma not excluded, but false positives are common in this range.  Exclude drug interference as a possible cause (see  www.newcastlelaboratories.com for a full list).  Consider measurement of plasma metanephrines after 30 minutes in a supine position (if not already done). |
| **Possible PPGL**  **2 to 3x ULRR** | NMET: 2361-3540  MET: 1021-1530 | Metanephrines in a range which suggests possible  phaeochromocytoma/paraganglioma.  Exclude drug interference as a possible cause (see  www.newcastlelaboratories.com for a full list).  Consider measurement of plasma metanephrines after 30 minutes in a supine position (if not already done). Consider discussion with an endocrinologist. |
| **Consistent with PPGL**  **>3x ULRR** | NMET: >3540  MET: >1530 | Metanephrines in a range consistent with  phaeochromocytoma/paraganglioma.  Consider urgent discussion with an endocrinologist. |

**Table 2.** Reference ranges and interpretation for seated patients (NMET = normetanephrine, MET = metanephrine, ULRR = upper limit of the reference range).

Local data indicates that around 20% of cases of phaeochromocytoma/paraganglioma (PPGL) are associated with a normetanephrine or metanephrine result between 1 and 2 times the upper limit of the reference range (ULRR), around 10% of cases 2 to 3 times the ULRR and around 70% of cases greater than 3 times the ULRR. Plasma metanephrines are susceptible to false positive results, typically in the borderline (1 to 2 times the ULRR) range so that results in this range, while not excluding phaeochromocytoma, are more frequently false positives than genuine cases of phaeochromocytoma (particularly in populations with a low prevalence such as patients with hypertension as the only suggestive feature).

4.2 SUPINE REFERENCE RANGES

If users wish to use supine sampling (after 30 minutes supine rest) for plasma metanephrines we recommend that appropriate reference ranges based on a supine reference population are applied (included in a separate comment on our reports). We do not have in-house supine reference ranges and so suggest that the following published reference ranges based on an LC-MS/MS method are used (5):

*Plasma normetanephrine:* <730 pmol/L

*Plasma metanephrine:* <450 pmol/L

*Plasma 3-methoxytyramine:* <180 pmol/L

4.3 PAEDIATRIC REFERENCE RANGES

We apply different reference ranges for patients under 1 year old, as normetanephrine and 3-methoxytyramine may be significantly higher in this age group (10):

|  |  |  |  |
| --- | --- | --- | --- |
| Paediatric ≤1 year | Plasma Normetanephrine  (pmol/L) | Plasma Metanephrine  (pmol/L) | Plasma  3-methoxytyramine  (pmol/L) |
| 6m to 1 year | <1440 | <510 | <245 |
| 3m to <6m | <2100 | <510 | <330 |
| <3m | <2540 | <510 | <420 |

1. **Follow-Up Testing for Borderline Results**

Although some cases of phaeochromocytoma are associated with plasma metanephrine results in the ‘borderline’ range (1 to 2 times the upper limit of the reference range), many results in this range represent false positives. There are several possible approaches when following-up borderline results.

5.1 SUPINE SAMPLING FOR PLASMA METANEPHRINES

There is a significant amount of published data to indicate that plasma metanephrines collected in the supine position after 30 minutes of supine rest offer improved diagnostic specificity compared to seated sampling (1). A lower false positive rate is therefore expected with supine sampling, potentially making this a useful follow-up test where plasma metanephrine results are borderline for samples taken in the seated posture. It should be noted that using reference ranges specific for supine individuals is recommended to maintain diagnostic sensitivity (see section 4.2).

5.2 MEDICATION-ASSOCIATED FALSE POSITIVES

A range of drugs have been associated with elevations in plasma metanephrines (see section 3.3 for more details). Briefly, some of the medications most commonly associated with false positive results are tricyclic antidepressants, phenoxybenzamine and beta-blockers. Exclusion of medication-associated false positives may be achieved by repeating analysis after potentially offending medications have been temporarily suspended, but this may not always be possible to carry out safely.

5.3 CLONIDINE SUPPRESSION TESTING

The clonidine suppression test has been reported as a possible follow-up test for discrimination of false and true positives (6). However, this has not been fully validated and we have no local experience of the test or diagnostic cut-off available.

5.4 CHROMOGRANIN A

Measurement of serum/plasma chromogranin A (CgA) has been discussed as a secondary test for phaeochromocytoma (7). Again, this remains to be fully validated and any diagnostically useful cut-offs will be assay-specific as there is poor agreement between different CgA assays.

1. **Clinical Significance of 3-Methoxytyramine**

7.1 3-methoxytyramine (3-MT) is the O-methylated metabolite of dopamine. An elevation in 3-MT may be the only abnormality in the plasma metanephrines (5,8) in the rare cases of phaeochromocytoma/paraganglioma that exclusively secrete dopamine. Another potential use of 3-MT is in the prediction of the presence of metastatic disease in phaeochromocytoma/ paraganglioma. In a study of 63 patients with phaeochromocytoma/paraganglioma (14 with metastatic disease) average concentrations of 3-MT were significantly higher in patients with metastases compared to those without (8). 3-MT is also higher on average in patients with mutations in SDHB and SDHD compared to other inherited susceptibility syndromes (8).

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