

## **PLASMA METANEPHRINES**

### **INSTRUCTIONS FOR USERS AND REQUESTING CLINICIANS**

#### **1. SAMPLE REQUIREMENTS**

- 1.1 EDTA whole blood samples are preferred (although heparin samples can be used).
- 1.2 Minimum sample volume: 1 mL EDTA whole blood
- 1.3 Minimum assay volume: 100 µL plasma

#### **2. COLLECTION PROCEDURES AND SAMPLE STABILITY**

- 2.1 Whole blood samples must 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.

#### **3. PATIENT PREPARATION AND POSTURE DURING SAMPLING**

##### **3.1 PATIENT POSTURE DURING SAMPLING**

Routine practice in Newcastle (and in most other UK centres) is to collect samples from seated patients for plasma metanephrines. The reference ranges used in our reports are based on a seated population. There are recently 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, a local study of the diagnostic performance of plasma metanephrines

using a protocol of seated sampling and seated reference ranges revealed high diagnostic sensitivity (100 % when three cases of non-functional paraganglioma were excluded) and specificity (90 %) using this approach (3). Utilising published supine reference ranges had no impact on diagnostic sensitivity in the group of patients studied but significantly impaired diagnostic specificity, so 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.

Although local data suggests that a seated sampling approach achieves high diagnostic sensitivity, supine sampling is considered to be the ideal sampling protocol, given the improved diagnostic performance that may be achieved (2). 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

some evidence that the vasopressor midodrine may interfere in 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 noradrenaline, i.e. tricyclic antidepressants, 'selective' serotonin reuptake inhibitors and serotonin/noradrenaline reuptake inhibitors. Other potential causes of false positive results include anti-hypertensive drugs (e.g.  $\alpha$ - and  $\beta$ -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 catecholamine or metanephrine 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
$\alpha$ -adrenergic receptor blockers	Phenoxybenzamine, doxazosin, indoramin
$\beta$ -adrenergic receptor blockers	Atenolol, labetalol, propranolol
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 metanephrine results.

#### 4. 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:

	<b>Adult Reference Range (&gt;16 years)</b>	<b>Borderline Up to 2X ULRR</b>	<b>Possible Phaeo 2 to 4X ULRR</b>	<b>Consistent with Phaeo &gt;4X ULRR</b>
Plasma Metanephrine (pmol/l) PMET	<510	511-1020	1021 - 2040	>2040
Plasma Normetanephrine (pmol/l) PNMET	<1180	1181-2360	2361-4720	>4720
Comments  * For drug causes see section 3.3	These results do not suggest the presence of a phaeochromocytoma	Borderline increase in (PNMET/PMET) but not in a range normally associated with phaeochromocytoma. Exclude drug causes*	(PNMET/PMET) in range which suggests possible phaeochromocytoma. Exclude drug causes* Consider discussion with endocrinologist	(PNMET/PMET) in range which is consistent with phaeochromocytoma. Suggest urgent discussion with endocrinologist

**Table 2.** Reference ranges and interpretation for SEATED patients.

Local data indicates that around 10 % of cases of phaeochromocytoma/paraganglioma are associated with a normetanephrine or metanephrine result between 1 and 2 times the upper limit of the reference range (ULRR), around 15 % of cases 2 to 4 times the ULRR and around 75 % of cases greater than 4 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.

## 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. 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 do not have specific in-house plasma metanephrine reference ranges for paediatric patients. Paediatric reference ranges are available for urine metanephrines (24 hour collections and overnight collections).

## 5. FOLLOW-UP TESTING FOR BORDERLINE RESULTS

Although some cases of pheochromocytoma 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 a number of 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) also shows promise as a secondary test for pheochromocytoma (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.

## 6. 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 pheochromocytoma/paraganglioma that exclusively secrete dopamine. Another potential use of 3-MT is in the prediction of the presence of metastatic disease in pheochromocytoma/paraganglioma. In a study of 63 patients with pheochromocytoma/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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## 8. REFERENCES

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