PLASMA 3-METHOXYTYRAMINE ASSAY USING LC-MS/MS (REFERENCE — 2014.02.03)

Notice of Assessment

December 2014

1 GENERAL INFORMATION

1.1 Requester: CHUM – Hôpital Saint-Luc

1.2 Application for Review Submitted to MSSS: January 16, 2014

1.3 Application Received by INESSS: June 10, 2014

1.4 Notice Issued: October 31, 2014

> **Note:**
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> This notice is based on the scientific and commercial information submitted by the requester and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with fully automated online solid phase extraction.

2.2 Brief Description of the Technology, and Clinical and Technical Specifications

The requester uses a classic LC-MS/MS technique with automated online extraction. The technique includes three steps: 1) sample preparation, 2) liquid chromatography (LC), and 3) analysis using tandem mass spectrometry (MS/MS).

Sample preparation involves purifying the sample by removing proteins and other substances it contains to prepare it for analysis. Various methods may be used to achieve this, the three most common being: solid phase extraction (SPE), liquid-liquid extraction (LLE), and protein precipitation (PP) [Adaway and Keevil, 2012]. The solid phase method is used by the requester[1].

The LC method separates molecules in a complex medium (serum or plasma) according to their physicochemical properties (molecular weight, hydrophoby, or other). The principle of LC involves the use of a liquid mobile phase and a solid stationary phase (on a column or thin layer). The composition of the liquid and solid phases varies according to the type of molecules to be purified.

Mass spectrometry is used to determine the mass of molecules present in a given sample. The measurement of mass depends on the deflection of molecules—ionized previously by an electric or magnetic field—which have a trajectory proportional to their mass and charge.

The mass spectrometer is composed of:

An ionization source to change the charge of the molecules and transfer them into the gas phase (e.g., electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or other).

1. Based on the information provided by the requester.

1 An analyzer used to separate the ions according to their mass-to-charge ratio (m/z).

Analyzers may be used sequentially. This describes mass spectrometry run in multiple dimensions (in this case, in tandem, therefore MS/MS). The first analyzer selects ions according to a specific m/z (purification). The purified ions are then fragmented in a collison chamber. A second analyzer measures the m/z of the fragments [Adaway and Keevil, 2012; Grebe and Singh, 2011].

2.3 Company or Developer

In-house method. The requester uses the classic LC-MS/MS technique with fully automated online solid phase extraction on a Symbiosis-Quattro Premier system. For internal quality control, the test is carried out in duplicate on two in-house plasma pools and two different samples from material previously used for external quality control. A calibration curve is included with each series of tests, as well as a quality control from pooled serum and commercial products. External quality control is ensured through the laboratory's participation in the Australian RCPA Chemical Pathology Quality Assurance Programs. The reference values for plasma 3-methoxytyramine are: < 0.17 nmol/L.

2.4 Licence(s): Not applicable.

2.5 Patent, If Any: Not applicable.

2.6 Approval Status (Health Canada, FDA)

Not applicable. Reagents are prepared in the laboratory. Commercial chromatography column and solid phase extraction cartridges.

2.7 Weighted Value: 126.94

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

All patients in whom there is a suspicion of pheochromocytoma (PHEO) or a paraganglioma (PGL) as well as patients with a germline mutation known to be associated with these tumours.

3.2 Targeted Disease(s)

Pheochromocytomas and Paragangliomas

PHEOs and PGLs are tumours that develop in neuroendocrine tissue (chromaffin cells) of neural crest origin, and belong to the sympathetic and parasympathetic nervous system [Rouaix-Emery et al., 2014]. The 2004 WHO classification of endocrine tumours defines PHEO as an intra-adrenal PGL [Pacak et al., 2011].

PGLs associated with the parasympathetic system develop primarily in the head and neck and are generally low-secreting [Rouaix-Emery et al., 2014], whereas those associated with the sympathetic system arise in the adrenal medulla or the sympathetic ganglia in the thoracic, abdominal, and pelvic regions, and are potentially active. The term "pheochromocytoma" usually refers to chromaffin

tumours of the adrenal medulla.

Both PHEO and PGL tumours are sporadic in 70% of cases or are involved in genetic predisposition syndromes in 30% of cases: multiple endocrine neoplasia (MEN), type 2A and type 2B (*RET* gene), von Hippel-Lindau disease (*VHL* gene), hereditary paraganglioma syndrome (*SDHA*, *SDHB*, *SDHC*, *SDHD* genes), and neurofibromatosis type 1 (*NF1* gene) [Rouaix-Emery et al., 2014]. The *SHDB* mutation, initially reported in a German and Polish cohort, is the most common mutation worldwide [Lefebvre et al., 2012] and primarily concerns extra-adrenal PGL, particularly of the head and neck [Pacak et al., 2011].

Clinical expression varies according to the tumour's location, size, and level of catecholamine hormone secretion. Certain catecholamines lead to sudden-onset high blood pressure associated with severe headaches, sweating, and palpitations—the classic presentation of an active, secreting PHEO. Some cases remain asymptomatic. Moreover, there is not necessarily a correlation between symptoms of hypertension and levels of circulating catecholamines [Rouaix-Emery et al., 2014]. High circulating levels of dopamine can cause hypotension [Koch et al., 2003].

Catecholamines include adrenaline (epinephrine), noradrenaline (norepinephrine), and dopamine. Catecholamines are synthesized from L-tyrosine in food or from the hepatic metabolism of phenylalanine. Dopamine is first converted into noradrenaline then into adrenaline enzymatically. The enzyme catechol-O-methyltransferase (COMT) transforms primary circulating catecholamines into metanephrines, which include normetanephrine, metanephrine and 3-methoxytyramine (3-MT), blood catabolites of noradrenaline, adrenaline, and dopamine, respectively [Peaston et al., 2010]. The vast majority of metanephrines (95%) undergo sulfo-conjugation in the gastrointestinal tract, and vanilmandelic (adrenaline, noradrenaline) and homovanillic (dopamine) acids are present in urine as a result of monoamine oxidase (MAO) enzymes and aldehyde dehydrogenase activity [Rouaix-Emery et al., 2014]. Free metanephrines reflect tumour secretion and account for 5% of total metanephrines measured in circulating blood [Rouaix-Emery et al., 2014]. Moreover, plasma levels of free 3-MT indicate a higher number of active PGLs than urinary excretion rates (33% versus 27%; $p < 0.05$) [Van Duinen et al., 2013].

According to the requester, these rare and potentially fatal tumours can affect adults and children (in 10% of cases) and have a prevalence of 1/30,000. In a recent publication, Rouaix-Emery et al. (2014) reported an incidence of 0.95 new cases per 100,000 population. The incidence is underestimated, as these diseases are poorly understood and underdiagnosed. A founder mutation occurring in the French-Canadian population predisposes to the development of PGL (SDHC) [Dumas et al., 2011].

3-Methoxytyramine Assay

Except for certain cases of paragangliomas secreting only dopamine and its metabolite 3-MT, the detection of these diseases (PHEO and PGL) does not require a 3-MT assay. However, once the diagnosis is established, 3-MT assays are included in the requested biochemical workup. Among other things, the assays facilitate the selection of genetic tests used to identify the mutation responsible for the disease. 3-MT assay provides an indicator of tumour malignancy, since dopamine-secreting tumours are more likely to be metastatic. The assay is also included in the follow-up

reports of patients with metastatic tumours, to rapidly detect relapses. Patients who develop the disease, as well as individuals who are carriers of the germline mutation (which increases the risk of developing the tumours), will receive an annual follow-up that includes tests for 3-MT.[2]

3.3 Number of Patients Targeted

From 10 to 15 new cases per year plus 15 to 30 follow-ups for metastatic cancers.

3.4 Medical Specialties and Other Professions Involved

Internal medicine, endocrinology, oncology, eventually ENT (tumours of the neck), genetics, pediatrics.

3.5 Testing Procedure

Plasma sample drawn by venipuncture after an overnight fast and a minimum period in the supine position and stored in a heparinized or EDTA tube [Rouaix-Emery et al., 2014; Peitzch et al., 2013; Lenders et al., 2007; Pacak et al., 2007]. Levels of 3-MT may increase after food intake—which is why it is important to fast beforehand [Van Duinen et al., 2013; De Jong et al., 2009]—, and after the use of tricyclic antidepressants or acetaminophen [Rouaix-Emery et al., 2014]. The intake of caffeinated and decaffeinated beverages should also be avoided [Darr et al., 2014]. Patients are strongly advised to stop taking any medications affecting catecholamine metabolism one week prior to blood testing,[3] if possible.

Moreover, one study did not identify any significant differences in the measurements of plasma 3-MT based on age, BMI,[4] and gender [Van Duinen et al., 2013], which is not the case for other metanephrines in the diagnosis of PHEO [Rouaix-Emery et al., 2014].

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

This test does not replace other tests such as blood and urine tests for catecholamines and metanephrines, but it is complementary; it specifically measures 3-MT, a circulating metabolite of dopamine. Therefore, this test enables the detection of all dopamine-secreting PHEOs and PGL, thus ensuring better patient management.

4.2 Brief Description of the Current Technological Context

According to the requester, the biochemical diagnosis of PHEO and PGL begins with assays of catecholamines and metanephrines in plasma and urine. Once the diagnosis has been confirmed, assaying 3-methoxytyramine will help guide mutation testing (genetic test options), if relevant, to identify the mutation causing the disease.

Currently available technologies include liquid chromatography coupled with electrochemical detection (LC-ECD) [Peitzsch et al., 2013; Eisenhofer et al., 1986], high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) [Pillai and Callen, 2010], enzyme immunoassays (EIA) [Pillai and

2. Based on the information provided by the requester, 2014.
3. *Ibidem*.
4. BMI: body mass index.

Callen, 2010], and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [Leung and Fong, 2014; Peitzsch et al., 2013].

4.3 Brief Description of the Advantages Cited for the New Technology

The LC-MS/MS technique is now widely used in clinical laboratories [Ackermans et al., 2014]. Its analytical specificity is superior to that of immunoassays [Pillai and Callen, 2010] and high-performance liquid chromatography (HPLC), and it has higher throughput than conventional GC-MS [Grebe and Singh, 2011]. It also has a higher specificity than that of HPLC-ECD and may be used to test smaller volumes. It is more cost-effective, since it reduces specimen processing and analysis time compared with HPLC-ECD [Van Berkel et al., 2014]. This analytical method eliminates chromatography interferences through structural analogy, but it does not eliminate pharmacological interferences, which is why it is important to obtain a history of patients' medications or to have them stop taking certain medications prior to blood testing [Rouaix-Emery et al., 2014].

It has recently become possible to conduct fully automated online solid phase extraction coupled with LC-MS/MS, which eliminates several limitations of offline solid-phase techniques, such as solvent evaporation, while providing the advantage of testing a larger number of samples [Peaston et al., 2010] and taking advantage of its speed (approximately 31 minutes with the method used by the requester).

4.4 Cost of Technology and Options: Not assessed.

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced

The 3-MT assay is complementary to assays of plasma and urinary adrenaline and noradrenaline metabolites in patients who have or are suspected to have dopamine-secreting PHEO or PGL. Eisenhofer et al. [2005] were the first to report the usefulness of measuring plasma free 3-MT for the detection and monitoring of dopamine-secreting PGL.

5.1.2 Diagnostic or Prognostic Value

3-MT assays will help better manage patients with PHEO or PGL, by screening out catecholamine-secreting tumours at low noise, which is not the case with the usual markers such as urinary catecholamines and plasma metanephrine and normetanephrine. The tumours are life-threatening and may also lead to cardiovascular and cerebrovascular accidents.

The only treatment that improves survival, in the case of PHEO or PGL, is surgical resection [Jimenez et al., 2013], which can be performed on small tumours without any extension, thus the importance of early detection of tumours and tumour relapses.

This has an impact on the treatment of patients with dopamine-secreting PGL. Therefore, according to the requester, this new test will provide a sensitive method of detecting response to surgery for metastatic disease and will be an essential tool in monitoring recurrences. Among patients with PHEO or PGL, the concentration of 3-MT was 4 to 6 times higher in patients with metastases than in those who did not

have any [Rouaix-Emery et al., 2014; Peitzch et al., 2013; Eisenhofer et al., 2012].

Moreover, serial 3-MT assays will allow early detection of primary tumours and relapse without the repeated use of extensive radiologic examinations, which have oncogenic effects.

Lastly, this test will allow patients with the disease to be assessed for genetic predisposition (particularly the *SDHB* gene, but also *SDHD*), which occurs in 30% of cases [Rouaix-Emery et al., 2014], and as high as 50% of cases when the disease is metastatic [Jimenez *et al.*, 2013]. Plasma concentrations of free 3-MT are more than 90-fold higher (*SDHB*) and more than 70-fold higher (*SDHD*) than reference values in the presence of PGL with these mutations [Eisenhofer et al., 2011b]. In addition to patients known to have the disease, carriers of the germline mutation that increases the likelihood of these tumours developing will be monitored. This applies particularly to cases involving the founder mutation (*SDHC*) that predisposed many members of French-Canadian families to the disease [Dumas et al., 2011].

5.1.3 Therapeutic Value: Not applicable.

5.2 Clinical Validity

COMPONENT	PRESENCE	ABSENCE	NOT APPLICABLE
Sensitivity	X		
Specificity	X		
Positive predictive value (PPV)		X	
Negative predictive value (NPV)		X	
Likelihood ratio (LR)		X	
ROC curve	X		
Accuracy	X		

Sensitivity and Specificity

The clinical sensitivity and specificity of 3-methoxytyramine (3-MT) tested using LC-MS/MS were 86% and 96%, respectively (see Table 1).

Table 1: Sensitivity, specificity, PPV, and NPV

STUDY	METHOD	NUMBER OF SAMPLES	SENSITIVITY (%)	SPECIFICITY (%)
Peitzsch et al., 2013	LC-MS/MS	63	86	96
Eisenhofer et al., 2012	LC-ECD Threshold value of 0.2 nmol/L	-	57	85

Abbreviations: LC-ECD = liquid chromatography coupled with electrochemical detection; NPV = negative predictive value; PPV = positive predictive value.

ROC Curve

Peitzsch et al. [2013] reported an area under the curve (AUC) of 0.902 for 3-MT, with a threshold value of 0.41 nmol/L.

Eisenhofer et al. [2012] reported an AUC of 0.716 ($p < 0.0001$) for plasma free MT, whereas the AUC was 0.547 ($p = 0.171$) for normetanephrine (NMN). Therefore, 3-MT is a better biomarker of malignancy than NMN. Moreover, when 3-MT is compared with the diameter of the tumour to predict the presence of malignancy, it is almost comparable, with an AUC of 0.739 ($p < 0.0001$) when the threshold value is 0.2 nmol/L compared with an AUC of 0.771 ($p < 0.0001$) for the diameter of the tumour. In the same study, the AUC indicates that, among the 18 catecholamine analytes tested, 3-MT is the most sensitive biomarker for patients with or without metastases.

Accuracy

In the study by Peitzsch et al. [2013], 3-MT correctly classified the presence or absence of metastases in 86% of patients ($F = 12.04$, $p = 0.0097$).

In the study by Eisenhofer et al. [2011a], 3-MT correctly classified: 53% of MEN2[5] and NF1[6] versus *VHL*[7], *SDHB*[8], and *SDHD*[9] and 78% of *VHL* versus *SDHB* and *SDHD*. When a combination of tests (3-MT, NMN, and MN[10]) is used, the percentage of correctly classified patients is almost 100% for MEN2 and NF1 versus *VHL*, *SDHB*, and *SDHD* and 78% for *VHL* versus *SDHB* and *SDHD*.

In the study by Eisenhofer et al. [2012], the likelihood of metastasis for adrenal tumours is < 10% if the concentration of 3-MT is normal and > 33% if the concentration of 3-MT > 3 nmol/L. For extra-adrenal tumours, the probability of malignancy is > 70% if the concentration of 3-MT is > 3 nmol/L.

5.3 Analytical (or Technical) Validity

COMPONENT	PRESENCE	ABSENCE	NOT APPLICABLE
Repeatability	X		
Reproducibility	X		
Analytical sensitivity	X		
Analytical specificity	X		
Matrix effect		X	
Concordance		X	
Correlation between test and comparator	X		
Other, depending on type of test			X

5. MEN2: multiple endocrine neoplasia type 2.
6. NF1: neurofibromatosis type 1.
7. VHL: von Hippel-Lindau.
8. *SDHB*: succinate dehydrogenase subunit B.
9. *SDHD*: succinate dehydrogenase subunit D.
10. NMN: normetanephrine; MN: metanephrine.

Two validation studies on the measurement of 3-methoxytyramine (3-MT) using LC-MS/MS were identified [Peitzsch et al., 2013; Peaston et al., 2010].

Analytical Sensitivity

The analytical sensitivity of LC-MS/MS for the detection of 3-MT is represented by the limit of detection (LOD) and lower limit of quantification (LLOQ) values (see Table 2). The LOD ranges from 0.02 nmol/L to 0.03 nmol/L, whereas the LLOQ ranges from 0.024 nmol/L to 0.06 nmol/L.

Repeatability and Reproducibility

The values of the intra-assay CV range from 2.9% to 11.5%, whereas those of the inter-assay CV range from 7.8% to 12.9% (see Table 3).

Table 2: Validation studies on the measurement of 3-methoxytyramine using LC-MS/MS

STUDY	NUMBER AND TYPE OF SAMPLES	VOLUME (µL)*	LC-MS/MS ANALYSIS						
			PREPARATION METHOD	INTERNAL CONTROL	IONIZATION	LOD (nmol/L)	LLOQ (nmol/L)	LINEARITY (nmol/L)	COEFFICIENT OF LINEARITY
Peitzsch et al., 2013	Plasma	-	SPE	3-MT-d_4	ESI in MRM mode (positive)	0.02	0.024	2.5 – 50	r = 0.999
Peaston et al., 2010	28 Plasma (free 3-MT)	- / 35	online SPE	3-MT-d_4	ESI in MRM mode (positive)	0.03	0.06	0.1 – 23	$r^2 > 0.99$

Abbreviations: 3-MT = 3-methoxytyramine; 3-MT-d_4: 3-methoxytyramine-α,α,β,β-d_4 (deuterated); ESI = electrospray ionization; LC-MS/MS = liquid chromatography coupled with tandem mass spectrometry; LLOQ = lower limit of quantification; LOD = limit of detection; µL = microlitre; MRM = multiple reaction monitoring; nmol/L = nanomoles per litre; SPE = solid phase extraction.

* Sample volume / volume of aliquot injected into the HPLC system

Table 3: Repeatability and reproducibility of LC-MS/MS measurement of 3-methoxytyramine

STUDY	NUMBER OF SAMPLES	NOMINAL CONCENTRATION (nmol/L)	INTRA-ASSAY CV (%)	INTER-ASSAY CV (%)
Peitzsch et al., 2013	Intra-assay: 12 Inter-assay: 40	0.102	5.6	11.4
		0.27	5.2	7.8
		3.23	2.9	8.9
Peaston et al., 2010	20 replicates	0.35	11.5	-
	20 replicates	1.25	9.1	-
	20 replicates	3.25	7.1	-
	20 assays	0.28	-	12.9
	20 assays	1.15	-	10.2
	20 assays	3.55	-	8.9

Abbreviations: CV = coefficient of variation; nmol/L = nanomoles per litre.

Analytical Specificity

The recovery values of 3-MT range from 66% to 83% [Peitzsch et al., 2013] or from 88% to 98% [Peaston et al., 2010]. However, the number of specimens and nominal concentration for these data are not mentioned in the publications. No interferences were observed.

According to one study, metanephrine and normetanephrine can induce substantial cross-reactivity or contamination in the chromatographic profile of 3-MT by LC-MS/MS with automated online extraction [Twentyman et al., 2012]. There is no mention of this in any other study.

Correlation between Test and Comparator

The study by Peitzsch et al. [2013] is the only study to have compared LC-MS/MS with LC-ECD on 80 plasma samples. The correlation coefficient *r* is 0.635 ($p < 0.0001$); it indicates that the two methods produce relatively similar results.

Concordance

Plasma concentrations of 3-MT were 26% lower when measured with LC-MS/MS than they were when measured with LC-ECD [Peitzsch et al., 2013].

Local Technical Validity Data

The method validation data provided by the requester are the following: a linearity in plasma of 0.048 nmol/L to 24.55 nmol/L; an LOD of 0.012 nmol/L; an LOQ of 0.048 nmol/L; an intra-assay CV of 3.7% to 7.7% (n = 10), and an inter-assay CV of 2.3% to 13.8% (n = 13) with concentrations of 0.15 nmol/L to 15 µmol/L; an accuracy of 105.7%, 100.1%, and 101.1% (for three external quality controls); a recovery of 93.2% of the 3-MT and an average matrix effect of 144.4%.

5.4 Recommendations from Other Organizations

Two documents focusing on recommendations for clinical practice were identified, but neither specifically refers to measurements of 3-MT. The first is a set of clinical practice guidelines developed by the Endocrine Society (United States) [Lenders et al., 2014], and the second is a set of recommendations produced during an international symposium on pheochromocytoma [Pacak et al., 2007].

These documents recommend that measurements of plasma free and urinary fractionated metanephrines (normetanephrine and metanephrine) be performed as first-line tests (as initial biochemical tests) for the diagnosis of PHEO and PGL. However, no references to 3-MT are made. Genetic testing will have to be considered in patients with germline mutations [Pacak et al., 2007]. Patients with PGL should be tested for succinate dehydrogenase (SDH) mutations, and those with metastatic diseases should be tested for *SDHB* mutations [Lenders et al., 2014]. Lifelong annual follow-up is recommended for the detection of metastatic or recurrent diseases [Lenders et al., 2014]. Therefore, the measurement of 3-MT might be of interest in guiding genetic testing, although no mention of this is made in the documents.

In a recent document, Eunice Kennedy Shriver NICHD, NIH, recommends that the initial workup for the diagnosis of PHEO and PGL include metanephrines and indicates that the measurement of 3-MT is preferable for dopamine-secreting tumours [Moraitis et al., 2014].

6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

The equipment required for testing with LC-MS/MS is already in place in most laboratories. Qualified personnel for this test should be provided if not already available.

6.2 Economic Consequences of Introducing Test Into Quebec's Health Care and Social Services System

Not assessed.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues

Patients known to have the disease, as well as carriers of the germline mutation, would eventually be followed annually with workup that includes 3-MT, although this may raise certain ethical issues. These patients should be encouraged to participate in the decision-making process for genetic testing [Lenders et al., 2014].

7 IN BRIEF

7.1 Clinical Relevance

This test is complementary to existing tests (plasma and urinary catecholamines, metanephrine, normetanephrine), as it specifically measures free 3-MT, a circulating dopamine metabolite. This measurement enables the detection of all dopamine-secreting PHEOs and PGLs, thereby ensuring better disease management. Better management includes early detection of dopamine-secreting tumours and monitoring to identify any recurrence or metastasis after surgical resection; but above all, it enables the assessment of genetic predisposition, present in 30% of cases. This is especially revelant for cases involving the founder mutation (*SDHC*) to which many members of French-Canadian families are predisposed.

7.2 Clinical Validity

The sensitivity and specificity of the test are 86% and 96%, respectively. 3-MT is a better biomarker of malignancy than normetanephrine. When it is compared with the diameter of the tumour to predict the presence of malignancy, it is almost equivalent. Moreover, 3-MT is the best biomarker for discriminating patients with or without metastases. 3-MT can correctly classify the presence or absence of metastases (accuracy) in 86% of patients , MEN2 and NF1 versus *VHL*, *SDHB*, and *SDHD* in 53%, as well as *VHL* versus *SDHB* and *SDHD* in 78%. When the concentration of 3-MT is > 3 nmol/L, the likelihood of metastases is > 33% for adrenal tumours, and the likelihood of malignancy is > 70% for extra-adrenal tumours.

7.3 Analytical Validity

Tests for 3-MT using LC-MS/MS show very high analytical sensitivity (LLOQ ≥ 0.024 nmol/L), coefficients of variation of < 12% (accuracy) and < 13% (reproducibility) as well as a recovery rate of 66% to 98% (analytical specificity). Only one study [Twentyman et al., 2012] referred to the possibility of cross-reactivity induced by metanephrine or normetanephrine during the test. The LC-MS/MS technique yields plasma concentrations of 3-MT that are 26% lower than those measured with LC-ECD. Local technical validation data show a variation of < 8% and < 14% for repeatability and reproducibility, respectively. The accuracy is almost 100%, with a recovery rate of approximately 93% and a matrix effect of approximately 144%.

7.4 Recommendations from Other Organizations

Two documents [Lenders et al., 2014; Pacak et al., 2007] pertaining to recommendations for clinical practice were identified, but neither specifically refers to assaying 3-methoxytyramine. However, 3-MT assays may be of interest in guiding genetic testing, although this also is not mentioned in the documents. A recent recommendation [Moraitis et al., 2014] emphasized the importance of adding to existing tests plasma free 3-MT assays for dopamine-dependent PGL.

8 INESSS NOTICE IN BRIEF

Measurement of Plasma 3-Methoxytyramine Using LC-MS/MS

Status of the Diagnostic Technology:

- ☒ Established
- ☐ Innovative
- ☐ Experimental (for research purposes only)
- ☐ Replacement for technology:_____which becomes obsolete

INESSS Recommendation:

- ☒ Include test in the Index, conditional upon the use of existing codes in the *Répertoire pour les catécholamines* (30112 and 30113)
- ☐ Do not include test in the Index
- ☐ Reassess test

Additional Recommendation:

- ☐ Draw connection with listing of drugs, if companion test
- ☐ Produce an optimal use manual
- ☐ Identify indicators, when monitoring is required

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