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### ► To cite this version:

Jean-Benoît J.-B. Corcuff, Laurence Chardon, Ines El Hajji Ridah, Julie Brossaud. Urinary sampling for 5HIAA and metanephrines determination: revisiting the recommendations. *Endocrine Connections*, 2017, 6 (6), pp.R87-R98. 10.1530/EC-17-0071 . hal-02628684

**HAL Id: hal-02628684**

**<https://hal.inrae.fr/hal-02628684>**

Submitted on 26 May 2020

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# Urinary sampling for 5HIAA and metanephrines determination: revisiting the recommendations

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## Abstract

**Context:** Biogenic amines such as 5-hydroxy-indole acetic acid (5HIAA) the main metabolite of serotonin or metanephrines (catecholamines metabolites) are used as biomarkers of neuroendocrine tumours.

**Objective:** To re-evaluate the recommendations for urinary sampling (preservatives, diet, drugs, etc.) as many of the reported analytical interferences supporting these recommendations are related to obsolete assays.

**Methods:** Bibliographic analysis of old and modern assays concerning preservation, extraction, assay and interferences.

**Results:** 5HIAA may degrade as soon as urine is excreted. Thus, acids as preservatives (hydrochloric or acetic acid) have to be immediately added. Care should be taken not to decrease the pH under 2. Urine preservative for metanephrine assays is not mandatory. Diets including serotonin-, tryptophan- and dopamine-rich foods have to be avoided depending on the biomarkers investigated (bananas, plantain, nuts, etc.). Tryptophan-rich over-the-counter formulas have to be prohibited when 5HIAA has to be assayed. Acetaminophen may interfere with electrochemical detection depending on high-pressure liquid chromatography (HPLC) parameters. No interference is known with mass spectrometric assays but with the one described for metanephrines determination. Some drugs interfere however with serotonin and catecholamines secretion and/or metabolism (monoamine oxidase inhibitors, serotonin or dopamine recapture inhibitors, etc.).

**Conclusion:** Revisited recommendations are provided for the diet, the drugs and the preservatives before HPLC coupled with electrochemical and mass spectrometry assays.

## Key Words

- ▶ 5-hydroxy indole acetic acid
- ▶ metanephrines
- ▶ assay
- ▶ neuroendocrine tumours

Endocrine Connections  
(2017) 6, R87–R98

## Introduction

Endocrine and neuroendocrine tumours diagnosis is based on the convergence of clinical, imaging and biological arguments. Tumour secretory markers are often hormones (or their metabolites) that are secreted by normal tissue as well as tumoural cells although frequently in an abnormal way (different level, loss of negative feed-back, unusual

molecular form, etc.). Many hormones or metabolites can be assayed in serum or plasma. Most assays are now modern immunoassays, mass spectrometry assays or electrochemical assays. Although prone to disadvantages such as cumbersomeness, incomplete collection and analytical matrix effect, urine collection provides an integrated profile



of the daily filtration of secreted hormone or metabolites. Thus, 24h urine sampling is still useful in endocrine tumours. Whatever the assays undertaken, pre-analytical steps are crucial to assure the relevance of the analysis. More specifically, exploration of urinary excretion requires some attention paid to sampling conditions: hormone or metabolite preservation in urine during the collection and transport, control of diet and environmental conditions, control of drugs altering hormone concentrations (secretion, recapture, metabolism, etc.), control of drugs altering the analyte assay, etc. Many endocrine-related assays in urine have been set up in the early days of modern endocrinology and relied on pioneer work on biochemical colorimetric or fluorimetric analysis. Obviously, techniques have changed replacing most of these assays by modern alternatives that are simultaneously more specific and more sensitive. However, in our clinical practice as well as in recently published international consensus about neuroendocrine tumours or pheochromocytomas or paragangliomas, we have encountered recommendations for urine sampling that were either clearly obsolete or not otherwise supported by bibliographic references (Supplementary Data, see section on supplementary data given at the end of this article). We thus collected these – often historical – recommendations and tried to sort out the ones that are still valid and those that are obsolete. We will try in this review to weed out the most out-dated recommendations for urine sampling considering the use of electrochemical or mass spectrometric detections coupled to high-pressure liquid chromatography (HPLC) and excluding colorimetric and fluorimetric assays. We will consider the preservation of analytes in urine, diet-related issues and drug-related issues. We will not discuss the level of diagnosis performed to quantify these analytes as this was covered in many publications.

### Urinary metabolite of serotonin, 5 hydroxy-indole acetic acid

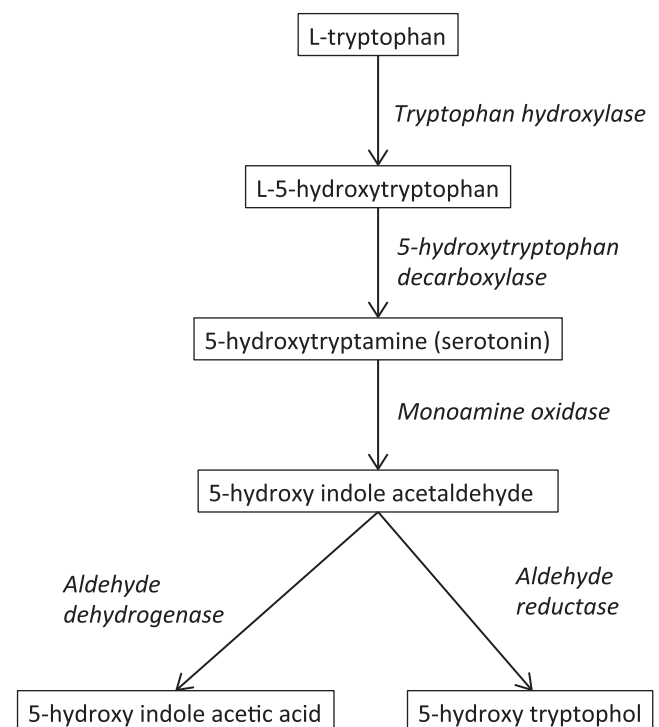
Because neuroendocrine cells may secrete serotonin, its main metabolite 5 hydroxy-indole acetic acid (5HIAA), or serotonin itself, may be used as tumour secretory markers to investigate and monitor patients with carcinoid syndrome (1, 2, 3, 4). Serotonin (5-hydroxytryptamin) is a monoamine neurotransmitter derived from the amino acid L-tryptophan via a 2-step enzymatic pathway (Fig. 1) (5). About 10% of serotonin is located in the neurons of the central nervous system. The remaining 90% is located in the enterochromaffin cells (5, 6). The 5HIAA is produced in the liver and excreted by the kidney.

Serotonin may be assayed either in its free state in the plasma or bound in platelets. 5HIAA can also be assayed in plasma but its daily excretion in urine is still the more classically recommended assay (1, 2, 7, 8). We will not consider here the respective level of diagnosis performance of serotonin vs 5HIAA determination. Strikingly, although consensus and review publications sometimes mention that most modern 5HIAA assays, if not all, provide recommendations for urine collection irrelative to modern assays. We will also mention new causes of false-positive causes of increased 5HIAA excretion.

### 5HIAA assays

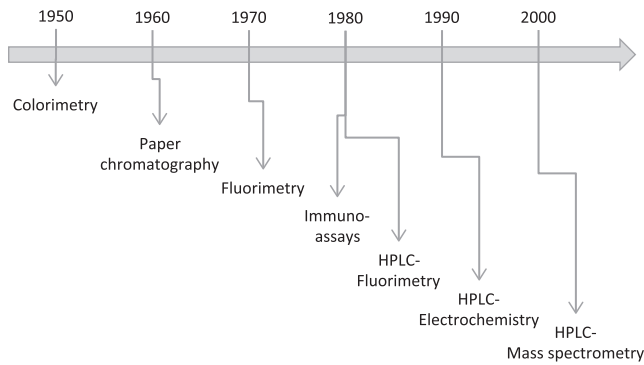
Historically, since these initial publications about 5HIAA and neuroendocrine tumours, assays have dramatically changed (Fig. 2). Assays in the 1950s were initially colorimetric (9, 10). Fluorimetric assays and gas chromatographic assays appeared in the 1960s (11, 12) followed in the 1980s by HPLC-fluorimetric assays (13, 14, 15) and immunoassays (16, 17).

Today, the most common assays use HPLC coupled to electrochemical detection (18, 19, 20). Recently, liquid



**Figure 1**

L-Tryptophan is metabolised by tryptophan hydroxylase, a rate-limiting step for serotonin synthesis. Monoamine oxidase occurs at 2 subtypes MAO-A and MAO-B with a widespread occurrence in the brain and in the periphery. They show differences in substrate specificities and sensitivities to inhibitors.



**Figure 2**  
Approximate chronology of the use of routine 5HIAA assays.

chromatography coupled to mass spectrometry has been used for the detection and quantification of urinary as well as serum 5HIAA (21, 22, 23). Obviously, these last 5HIAA assays after HPLC improved both specificity and sensitivity. Since the biochemical and biophysical reactions involved are very different, cross-reacting substances in colorimetric assays or fluorimetric assays have no systematic deleterious consequences on electrochemical detection or mass spectrometry. Conversely, new drugs may interfere with these modern assays especially with electrochemical detection.

### Urine preservation

Common sense advice for urine collection has to be given: collection in clean and closed plastic containers and complete collection of urine during a 24-h period. With no treatment, 5HIAA tends to degrade during and after a 24-h urine collection. To avoid an underestimation of its concentration preservatives have to be added to urine. Ideally, the pH should be lowered around 3 as early work stated that in an alkaline solution 5-HIAA is degraded. Hence, it is recommended to lower the pH of urine (usually with hydrochloric acid) but not lower than 3 as very low pH increases 5HIAA degradation (24).

Historically boric or acetic acid may be added to acidify urine. Acetic acid was erroneously described as inefficient as a preservative as, in fact, it caused problems with a one particular colorimetric assay. When acidified and kept at 4°C, 5HIAA is stable for more than one month (24). Various laboratories propose alternatives to acidification although published results are scarce. A useful list of preservatives for many analytes has been proposed (25). These alternatives to strong acids or boric acid (recently classified harmful and toxic for reproduction (26)) are especially useful for protecting patients and professional

from the preservatives themselves. Preservatives such as  $\text{Na}_2\text{CO}_3$  (although increasing pH) or  $\text{NaHSO}_3$  or EDTA are safe and easy to manipulate, and are recommended by some laboratories such as the Mayo Clinics (25). However, they have to be tested for each laboratory analytical procedure to ascertain both their real efficiencies and their absence of impact on the chosen assay. This is particularly important as solid-phase extraction of 5HIAA from urine may depend on pH. There is no difference of preservation when the pH is lowered to 3 or 1.5 with hydrochloric acid (our unpublished results). However, obviously to decrease pH to 1.5 a larger volume of acid has to be added. We also found that adding acetic acid as a preservative was not practical because the volume necessary to lower the pH to 3 resulted in an important dilution of urine. Despite being safe to manipulate, we ruled out  $\text{Na}_2\text{CO}_3$  as it prevented 5HIAA degradation only partly. Although some laboratories recommend protecting 5HIAA from light, we were not able to find many published data about this point. It has been related that as 5HIAA solution changes colour with light, a chemical reaction may occur (27). Although many recommendations advise to keep samples in the dark, we did not find any effect of light when hydrochloric acid was added as a preservative (unpublished results). Many laboratories also recommend keeping urine at 4°C during collection with the preservative. To our knowledge, there is no published data reporting the degradation of 5HIAA in acidified urine at room temperature: once urine was acidified, there were minor differences (5%) between room temperature and 4°C (unpublished results). Conversely, when a delay before analysis is unavoidable, it is safe to recommend refrigerating acidified urine samples.

### Diet-related issues

5HIAA is a metabolite issued from serotonin metabolism, and the latter is synthesized from the essential amino acid tryptophan (Fig. 2). Serotonin is mainly metabolised by monoamine oxidase in the liver and the lungs. Thus, diets rich in tryptophan (28) or serotonin (29) may increase 5HIAA urinary excretion. This point has been clearly established since it was believed that serotonin might be a remedy for constipation (30)!

Bananas and plantains were the first-described serotonin-rich fruits (30, 31, 32, 33); kiwi and pineapple have also high contents of serotonin (32, 33). Many (walnut and hickory families) but not all (chestnuts and pistachios) nuts have high serotonin contents. Almond,

cashews and macadamia nuts have intermediate contents. For example, walnuts contain twenty-fold more serotonin than bananas (34). Tomatoes and some varieties of plums and avocados have moderate serotonin contents (<5 µg/g) (32, 33). Strawberries, cranberries, raspberries, grapes, oranges apples and pears have apparently no detectable levels of serotonin (33). Eggplants were in an early study (10) reported to be rich in serotonin but not in a later one (32) suggesting that the former study used a less specific assay for 5HIAA determination.

Serotonin-rich fruits are also usually tryptophan rich although nuts have only twice the tryptophan contents of bananas (34). Seeds and nuts and beans are also usually tryptophan rich. Animal-derived products such as meat, milk, cheese may also have elevated tryptophan contents. The USDA provides a large Nutrient Database including one about tryptophan food content (35). It must be noted that all the ingested tryptophan is not found in urine as 5HIAA as tryptophan may be used as a structural amino acid and not solely as a precursor of serotonin. The publications reporting a follow-up of urinary excretion of 5HIAA usually claim a return to basal level two days after the end of the diet enriched either in tryptophan or serotonin (36).

Nowadays, many over-the-counter formulas contain tryptophan either alone or in combination with other compounds including 5-hydroxytryptophan. It is difficult to precisely indicate the subsequent increase of urinary 5HIAA because of the various tryptophan dosages available. These formulas should be discontinued before any biological investigation of neuroendocrine tumours as they can cause up to tenfold increase of urinary 5HIAA (37). Of note, the effect of dietary intake of 5 hydroxy-tryptophan on 5HIAA excretion seems very variable between individuals (37, 38). Serotonin is hardly present in over-the-counter formulas since it does not significantly cross the blood brain barrier and that most of these formulas target the brain alleging neuropsychological benefits. Lastly, dietary NaCl restriction increases 5HIAA excretion (39).

We encountered recommendations forbidding natural vanilla and vanillin during 5HIAA collection. We found no publication supporting this view and, conversely, Odink and coworkers reported no influence of vanilla on 5HIAA excretion (40).

### Drugs interfering with serotonin metabolism

Serotonin is a major neurotransmitter involved in nearly all neuronal pathways involving human behavioural

processes despite being produced by about one million neurons only (5). Because of this critical role, many drugs in psychiatric treatments target serotonin pathways. Furthermore, drugs targeting other pathways interfere with serotonin pathways because of its ubiquitous role. Several classes of drugs directly target the serotonin system: antidepressant, antipsychotic, anxiolytic, antiemetic, antimigraine drugs. Amongst these drugs, monoamine oxidase inhibitors and inhibitors of serotonin reuptake at the synaptic level should modify 5HIAA excretion. However, the effects may not be so clear as not all monoamine oxidase-inhibiting hydrazines have the same effect (41), suggesting that there may be some confusion of causes between the effects of the drugs on neurotransmitter pathways and interferences in earlier assays. Subsequently, however, many such drugs have been prohibited during 5HIAA collection for neuroendocrine tumours evaluation. For instance, the serotonin receptor antagonist risperidone mildly but significantly increases plasma 5HIAA (42). In rats, a reserpine analogue clearly increases 5HIAA excretion but the dosages far exceeded those used in humans (43). In rats or mice, many drugs either locally or generally administered alter 5HIAA concentrations in some brain structures: diazepam, phenobarbital, piperazine, methamphetamine... (44, 45, 46, 47). In humans, desipramine and zimeldine mildly reduced 5-HIAA concentrations in the cerebrospinal fluid (CSF) (48). Isoniazid given for depression was also suspected of modifying 5HIAA concentrations (49). This was probably because it was erroneously thought to be a monoamine oxidase inhibitor (50).

To summarize, to our knowledge, no report has been published about a major modification of 5HIAA urinary excretion in humans under psychiatric treatments. Of note, it must be kept in mind that some psychiatric diseases may also be associated to a modified serotonin metabolism. For instance, some autistic subjects display mildly increased 5HIAA excretion (51). In psychotic-depressed subjects, 5HIAA excretion was linked to insomnia and interest (52). Various studies of patients with depression reported differences in 5HIAA concentrations in CSF although they did not report urinary 5HIAA levels. 5HIAA concentrations in CSF, serum or urine are not modified by schizophrenia (53).

Thus, although possible, there is no hard data showing that these psychiatric drugs are really responsible for false diagnosis of neuroendocrine tumours. Of course, it may be because their discontinuation was often recommended! It may also be because the amount of



serotonin involved is low and concerns 'only' one million neurons compared to the much larger mass of serotonin in the neuroendocrine cells of the gut. Thus, although these drugs, and the disease they were prescribed for, may not be completely devoid of effect on 5HIAA excretion, it is difficult to rapidly and/or easily stop the treatments if they are necessary for psychiatric diseases. Should 5HIAA excretion under such treatment be over the upper limit of reference values, one should then consider interrupting the current psychiatric treatment.

### Drugs described as interfering with various techniques of 5HIAA quantification

The drugs described hereafter have been historically forbidden in various recommendations about 5HIAA urinary collection. *Guaifenesin* (glyceryl guaiacolate ether) has been used as a cough suppressant and as an expectorant. Since 1970, it has been known to cause errors in laboratory determinations of 5HIAA using 1-nitroso-2-naphthol (54). However, this interference should have been eliminated in 1972 by using a modified colorimetric technique (55). *Methocarbamol* and *mephenesin carbamate* elicited false-positive tests for 5HIAA (56). This happened because their urinary metabolites produce a positive nitrosonaphthol reaction similar to the one elicited by *Guaifenesin*. *L-DOPA* also gave false 5HIAA results in colorimetric reaction (57). *Homogentisic acid and/or gentisic acid* produced an artefactual increase in 5HIAA determination using a colorimetric assay in a patient with alcaptonuria (57). *Phenothiazine* and some of its derivatives have been described as falsely reducing 5HIAA urinary excretion. This is due to an interference in the development of colour in the colorimetric assay (58). This problem was partly corrected in the 70s in a modified colorimetric assay (59). *Aspirin* gave false 5HIAA results in a colorimetric assay (57). An improved method corrected this interference (60). *Antipyrin* interference was however not eliminated from this colorimetric reaction. *Aspirin* also gave false 5HIAA results in a fluorimetric assay (61). *Sulfasalazine*, *acetyl-5-aminosalicylic acid* and *mesalazine* mainly used for the treatment of Crohn's disease, ulcerative colitis and rheumatoid arthritis interfered with fluorescent assays (62). *Naproxen* falsely generated elevated 5HIAA urinary concentrations (63, 64). One of its metabolite reacted in a 5HIAA spectrophotometric assay (65).

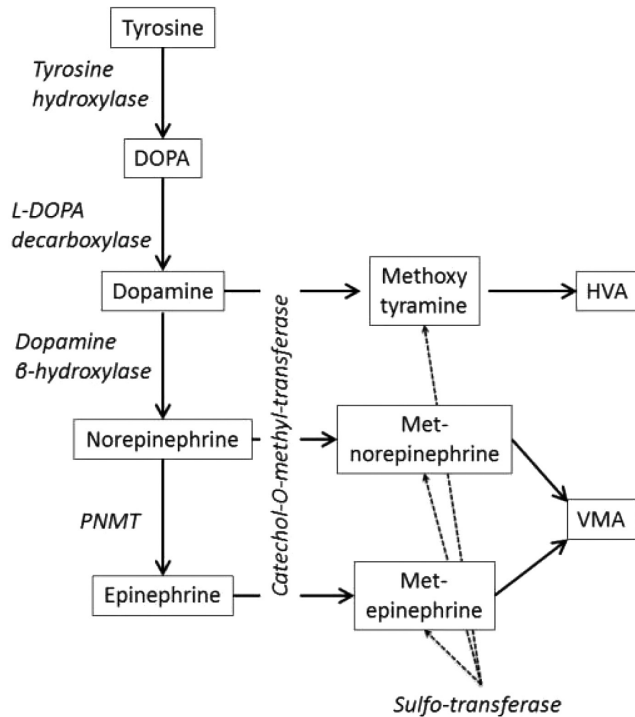
Very importantly, for all the above-mentioned compounds, there is no indication of interference when using electrochemical or mass spectrometric assays.

Nowadays, *paracetamol* (a.k.a. acetaminophen, 4-hydroxy acetanilide), a very common drug, may frequently be taken in investigated patients. Paracetamol has been the origin of one of the most serious electrochemical interferences when using oxidase-based amperometric biosensors. It produced an interfering current that increased glucose readings in early glucose sensors (66). In fact, *Paracetamol* can be assayed by electrochemical detection (67). Thus, it has been described as a potential source of interference in electrochemical detection after HPLC separation of analytes such as 5HIAA, metanephrines and catecholamines (68) or vanilmandelic and homovanillic acids (69) or serotonin (70) depending on the analytical method used (mobile phase, column, etc.). It could interfere with the peak of internal standard leading to overestimation of its value and, thus, lowering the value of the 5HIAA in the sample. Quality control of the internal standard signal should prevent this error. Of note, *Paracetamol* could, at least in rats, inhibit an enzyme metabolising tryptophan thereby reducing urinary 5HIAA (71).

### Urinary metabolites of catecholamines, metanephrines

To biologically diagnose pheochromocytomas or paragangliomas or neuroblastomas, various tumour-related markers might be assayed (72, 73, 74). Because these tumoural cells are from the same origin as the medullary adrenal, they may also secrete catecholamines and/or their methoxylated metabolites, a.k.a. metanephrines. Thus, catecholamines and metabolites may be used as tumour secretory markers either in plasma or urine.

Catecholamines are hormones derived from the amino acid tyrosine via a multistep enzymatic pathway (Fig. 3). Part of circulating catecholamines comes from neurons and part comes from the adrenal medulla (75): adrenaline originates from the adrenal medulla and noradrenaline comes from the adrenal medulla and the sympathetic nerves (75). Physiologically, metabolites are produced in catecholamine-synthesizing tissues from leakage of storage vesicles as well as in the liver to be excreted by the kidney and to a lower extent liver (see reviews (75, 76, 77)). Most metanephrines in urine are either sulfo- or glucurono-conjugated; free metanephrines in the plasma have half-lives lower than five minutes. Although it simplifies the assays if free catecholamines are simultaneously assayed (78),



**Figure 3**

Tyrosine is metabolised by a hydroxylase in dihydroxyphenylalanine (DOPA) that in turn is decarboxylated in dopamine. Dopamine can be hydroxylated in noradrenaline that in turn can be methylated by Phenylethanolamine N-Methyltransferase (PNMT) to form adrenaline. Dopamine, noradrenaline and adrenaline are inactivated by methylation forming metanephrines that can be sulphated or further metabolised by monoamine oxidases in homovanillic and vanillylmandelic acids (HVA and VMA, respectively).

there is no clinical argument (yet?) favouring the assay of free metanephrines in urine vs total metanephrines (although there are solid arguments in the plasma). Very importantly, in pheochromocytomas intratumoural metabolism generates metanephrines that are excreted by the tumour itself even though it does not excrete clear excess of catecholamines (76). This is why determining metanephrine levels either in urine or plasma is more clinically efficient than determining catecholamines level. Because of the latter and because they have been detailed elsewhere (79), we will not describe procedures for catecholamines collection as much detail as other analytes.

### Catecholamines and metabolites assays

The historical progress of catecholamines and metabolites assays followed the same course as 5HIAA assays did. For instance, the first chemical assays for catecholamines and metabolites were colorimetric (80) followed about fifty

years later by fluorimetric assays (81). In the late 60s, radioenzymatic assays were developed (82).

Electrochemical assays after liquid chromatography were developed in the late 80s using the same principles as colorimetric assays i.e. the ability to be oxidized. A prior high-pressure chromatography allows the separation of the different amines (83). In some laboratories, gas chromatographic assays and immunoassays were developed but they are less convenient. More recently, liquid chromatography coupled to mass spectrometry has been used for the detection and quantification of urinary catecholamines (84). Most authors assay total metanephrines i.e. after acid hydrolysis or enzymatic treatment of conjugated metanephrines. The first metanephrines assays (85) were progressively improved (86, 87, 88) but remained prone to analytical interferences compared to the more recent electrochemical assays. Some analytical interference is still occurring with the latter (89). Similar to other amines, liquid chromatography coupled to mass spectrometry has been recently developed with very rare interferences (90, 91).

### Urine preservation

Catecholamines and metabolites assays are very similar to 5HIAA assays and hence some technical recommendations can be identical. Complete urine collection should be performed in clean and closed plastic containers during a 24-h period.

Catecholamines are easily degraded unless preservatives such as HCl are added. However, in acidified urine, free catecholamines are less degraded but conjugated catecholamines are deconjugated. As the latter are in much higher concentrations, acidification may artefactually increase free catecholamines concentrations (92, 93). Thus, should catecholamines be required, moderate acidification is recommended (pH≈4).

Urinary metanephrines are much less degraded with time than catecholamines at least within a week (94). However, at 30°C, the degradation is not nil but can be prevented by HCl (Na<sub>2</sub>-EDTA is less efficient) (93). Thus, it is safe to add HCl if urine is not to be brought immediately to the laboratory. One point may be worth mentioning is that HCl, even at room temperature, may hydrolyse a fraction of conjugated metanephrines. Should free (and not total) urinary metanephrines be assayed, it would probably be better not to add HCl to minimise the risk of artefactually increasing the free fraction of metanephrines.

Homovanillic and vanillylmandelic acids (HVA and VMA, respectively) are historically collected with the same preservatives as catecholamines although they are more stable.

### Diet-related issues

Catecholamines (mainly, but not exclusively, dopamine) are present in a large variety of foods: fruits (especially bananas and plantains), nuts, tomatoes, beans, and cheeses (90, 95). These catecholamines are largely conjugated in the digestive tract by a sulphotransferase, SULT1A. Having a catecholamine-rich diet has then major consequences when total catecholamines are assayed in urine. Moreover, free noradrenaline and free dopamine are also increased by such a diet. Should catecholamines to be assayed be collected, it is then recommended to avoid these dietary products. One point worth mentioning is the existence of xenobiotics inhibiting SULT1A. SULT1A inhibition does not create the catecholamines, but prevents catecholamine deactivation (96). This could increase catecholamines and possibly metanephrines level from dopamine of gut origin whether endogenous or exogenous.

Metanephrines have not been reported to be present in food but part of the catecholamines ingested is to be metabolised into metanephrines (and further in HVA and VMA). Subsequently, conjugated metanephrine and normetanephrine and methoxytyramine (both in urine and plasma) increase after a catecholamine-rich diet (90). Free metanephrine and normetanephrine are not modified by such a diet but free methoxytyramine is increased (90). This latest point is important when screening for paragangliomas. HVA is mainly a metabolite of dopamine. Thus, as previously said, dopamine-rich diets have to be avoided before its assay in urine. Although less dependent on dopamine-rich diet, VMA excretion is still influenced by catecholamine intake. As for neuroblastomas, VMA and HVA are often requested in the same samples, and it is simpler to avoid these diets before urine collection for both assays. Diets that include artificial vanilla flavouring have to be avoided for VMA determination in urine because VMA is a chemical precursor of vanillin in food industry (an early assay used to transform VMA in vanillin to assay the latter!).

One natural component of Indian curry, *methoxyhydroxybenzylamine*, has been described as causing interference in HPLC with electrochemical detection for the detection of the internal standard using a commercial kit for metanephrines determination (97).

### Drugs interfering with catecholamine metabolism

Catecholamines are metabolised mainly by catechol-O methyl-transferase and aldehyde dehydrogenase (Fig. 3). Drugs inhibiting these enzymes such as *entacapone*, *disulfram* and possibly metronidazole have then to be avoided before catecholamines or catecholamines metabolites assays. Alcohol is also degraded by aldehyde dehydrogenase, and it has been reported that alcohol intake may interfere with catecholamines metabolism especially in subjects with allelic variations such as those of Asian descent (98). Thus, it may be recommended to suppress large alcohol intake during urine collection for catecholamine metabolites determination.

It should be kept in mind that many drugs although not interfering with the assays of metanephrines or catecholamines do interfere with the secretion of catecholamines. Most of them are drugs given for hypertension ( $\alpha$ 1- and  $\beta$ -adrenoceptor blockers) or neurological diseases (monamine oxidase inhibitors, tricyclic antidepressants, noradrenaline synaptic uptake such as antidepressant or cocaine) (79, 99). Lastly, acutely stressful situations (congestive heart failure, hypernoradrenergic hypertension, shock, sepsis, panic disorder, etc.) but also chronic pathologies such as obstructive sleep apnoea and metabolic syndrome increase catecholamine secretion (for review (79)). These points have to be taken into account when investigating a possible pheochromocytoma even – or possibly despite – using the best assay available.

### Drugs described as interfering with various techniques of catecholamines or metabolites quantification

Early colorimetric or fluorimetric assays exhibited many drug-elicited interferences (100, 101, 102, 103, 104, 105). These drugs should not cause interference with electrochemical or mass spectrometric assays.

Nowadays, as previously mentioned, *paracetamol* may interfere with electrochemical detection including the assays of catecholamines and metanephrines (68). *Methenamine*, a drug used in the prevention and treatment of urinary tract infections, altered the signal of the internal standard thus eliciting erroneously elevated metanephrines level (106). We described an interference with normetanephrine determination due to an antihypertensive drug, *urapidil* in an HPLC with electrochemical detection assay (107). *Amoxicillin* given in two paediatric patients caused



**Table 1** Main elements of urine collection for 5HIAA, metanephrines or VMA-HVA determination.

Analyte	Diet	Preservative	Drugs	Analytical interferences*
5HIAA	Banana, plantain, pineapple, kiwi, plums, walnut, hickory nut Tryptophan-containing supplements	Yes (HCl)	Inhibitors of monoamine oxidase and of serotonin reuptake; serotonin receptor antagonist	Paracetamol
Metanephrines	Banana, plantain nuts, tomatoes, beans	Not mandatory	Sympathomimetics, adrenergic receptor blockers, monamine oxidase inhibitors, noradrenaline uptake inhibitors, tricyclic antidepressants	Paracetamol, methenamine, urapidil, amoxicillin, buspirone, mesalamine, sulphasalazine
VMA-HVA	Banana, plantain nuts, tomatoes, beans; Artificial vanilla flavour (VMA)	Yes (HCl)	Sympathomimetics, adrenergic receptor blockers, monamine oxidase inhibitors, noradrenaline uptake inhibitors, tricyclic antidepressants?	Paracetamol

interference in a commercial normetanephrine assay (108). Another interference in the quantification of normetanephrine has been reported, in a mass spectrometry assay, because of a metabolite of methylenedioxy-methamphetamine *a.k.a. ecstasy* (109). Indeed, even mass spectrometry may present interference mainly with internal standard detection. In patients taking *L-DOPA* for Parkinson disease, 3-*O*-methyldopa, the methoxylated metabolite of the drug, might cause interference with measurements of methoxytyramine (110). Ibuprofen has been reported to interfere with the detection of HVA by gas chromatography with flame ionization detection after extraction and derivatization (111).

## Conclusion

Modern assays for the determination of the excretion of urinary 5HIAA and metanephrines are electrochemical or mass spectrometry assays. Molecules are detected after HPLC to sort the molecules in both techniques. Electrochemical quantification is very sensitive but interferences may occur (e.g. *paracetamol*). Mass spectrometry now achieves high sensitivity along with its high specificity (although for a higher cost). Subsequently, some ancient recommendations for urine collection are thus obsolete.

Although our purpose here was not to compare urine vs plasma samplings, it must be noted that because of these increases of specificity and sensitivity, assays in the plasma have been described for 5HIAA, metanephrines and methoxytyramine, VMA and HVA (72, 73, 112, 113, 114, 115). It is possible that these plasma assays will supplant urine assays for tumour assessment. Yet these

assays have not all been tested for the diagnosis of (neuro) endocrine tumours. It is important to note that although blood sampling during a consultation is very convenient compared to 24-h urine collection, it must be proven clinically par with urine collection. As the techniques for plasma assays are either electrochemistry or mass spectrometry, the recommendations will probably be close to those described for modern urine assays: diet, sample preservation, associated drugs, etc. Such recommendations have, for instance, been proposed for plasma metanephrines and methoxytyramine (90, 116, 117).

Meanwhile, in many countries, laboratories cannot invest immediately in the up-to-date analytical equipment to transform all their urine assays into plasma assays. Thus, for assays in urine, pre-analytical critical considerations are necessary to insure relevant data and conditions for evaluating urinary excretion have to be rigorous (Table 1). First, a complete 24-h urine collection coupled with good preservation has to be provided especially for 5HIAA determination (pH 3–4). Failure to insure these points may obviously result in false-negative tests. Secondly, oral intake of precursor amino acid such as tryptophan and of the amines themselves such as serotonin or dopamine has to be limited. Some specific fruits (bananas, plantains, nuts) beans and some cheeses as well as tryptophan-rich formulas should be prohibited two days before urine collection depending on the analysis to be done. Thirdly, paracetamol may have to be avoided in some electrochemical assays. Fourthly, drugs involved in serotonin or catecholamines metabolism or specific clinical circumstances such as stress have to be taken into account when analysing the results of a screening test. Interrupting psychiatric drugs involved in serotonin metabolism may prove necessary.

If necessary, drug-free collections may be necessary, and repeated sampling may be used because of the known variable metabolisms and diet intakes.

#### Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EC-17-0071>.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

#### Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

#### Acknowledgements

The authors wish to thank their colleagues from the Groupe de Biologie Spécialisée of the Société Française de Médecine Nucléaire for sharing their own experience and recommendations.

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Received in final form 3 May 2017

Accepted 30 May 2017

Accepted Preprint published online 31 May 2017

