MetBioNet urine amino acid requesting: a survey of current practice

Background

A recent audit of urine amino acid (UAA) requesting at the Evelina Children’s Hospital London showed that most requests were by non-specialists and were inappropriate [1]. However the lack of published standards made it difficult to establish evidence-based protocols for UAA requesting. Prior to this, the MetBioNet amino acids working group performed a review of all amino acid disorders and determined which required urine amino acids for diagnosis, recommending that UAA be performed in only a specific group of patients [2].

Establishing clear guidelines for the investigation of inherited metabolic disease (IMD) and providing advice on best practice could assist Clinical Biochemistry Departments, particularly non-specialist laboratories, in demand management and avoid unnecessary requesting of UAA analysis. Evidence of demand management is a CPA requirement. It is important that MetBioNet laboratories show a unanimous approach in light of specialist commissioning arrangements.

In order to do this information on the current practice in metabolic laboratories in the UK and Ireland was sought with the aim of establishing a consensus of opinion.

The Survey

MetBioNet stakeholders agreed to perform a review of current practice and to provide data from a 6-month period. A survey was prepared, peer-reviewed and then sent to 19 MetBioNet laboratories who were asked to forward it to other laboratories in their areas known to be performing UAA analysis. Responses were received from 15 MetBioNet and 3 other laboratories.

Response to the survey

The data obtained from the survey is presented in detail below.

The responses demonstrated very wide ranging practices and widely differing opinions of the value and use of UAA analysis. The differences were not based on laboratory size, workload or whether or not it was a MetBioNet stakeholder. The responses also highlighted limitations of the questionnaire, with some confusion over the questions particularly those asking for opinions.

There was overwhelming agreement that urine amino acids should be analysed to investigate patients with renal stones, renal tubular disease (both primary and secondary to other IMD including mitochondrial disease) and some respondents suggested specific conditions which require UAA analysis including hypophosphatasia and sulphite oxidase deficiency.

There was, however, considerable difference in practice and opinions on the use of UAA analysis in investigation of other IMD from those who state that it is an essential investigation to those who screen all requests and do not do UAA unless the request is for one of the disorders outlined above.

Some additional points of interest from the survey:

- Laboratories which vet requests perform substantially fewer UAA analyses and have more requests with clinical details than those which don’t vet them.
- The responses demonstrated a high proportion of requests accompanied by clinical information. However, some respondents stated that this information was not of a high enough quality to advise on appropriate selection of investigations.
• Two cases of homocystinuria were missed on UAA analysis during the 6-month period studied, confirming previous findings that urine homocystine shows poor diagnostic sensitivity for this disorder [3].

• One lab reported use of UAA to identify an unusual dietary pattern which initially was masked as an IMD.

• One lab reported reducing its workload by over 90% by introducing a protocol for selecting requests for UAA analysis and a fine for requestors who do not provide clinical information.

Conclusions

There was an excellent response to the survey which may reflect the interest in UAA and the need for laboratories to manage their workload.

The responses to the survey showed no consensus of opinion on the use of UAA in the investigation of IMD. This may in part be due to ambiguity over the questions used in the survey, but despite this there appears to be a clear difference of opinion between laboratories.

However, there is evidence that vetting requests results in a substantially reduced workload with one laboratory reporting a reduction in workload of 90% by introducing a protocol for requesting.

There is more work to be done to establish national guidance on the use of UAA, but the fact that approximately 50% of respondents use their own protocols should enable some evidence-based advice to be produced.

Further work

A guideline to be prepared based on data and experience from laboratories which vet requests to include conditions where UAA is an essential investigation, advice that UAA is not an essential in the first line investigation of IMD and suggested alternative investigations.

This will be used to educate non-specialist users of MetBioNet laboratory services and as a basis for reviewing requests.

A agreed statement will be prepared to go on bounced requests which outlines caveats and the need for good clinical information.

References


Acknowledgements

Thank you very much to the Clinical Biochemists who helped with preparation of the survey and to everyone who completed it with such enthusiasm!
MetBioNet urine amino acid requesting: A summary of responses

General
18 laboratories responded to the survey; 15 of 19 MetBioNet laboratories (79%) and 3 non-MetBioNet laboratories. All laboratories stated that they performed urine amino acids (UAA) analysis.

Processing requests for urine amino acid analysis

Q3. Does your laboratory vet requests for urine amino acid analysis?

Of the 8 labs that vet requests:
- 6 labs use clinical details (i.e. transport disorders, renal stones)
- 1 lab uses the creatinine cut off
- 1 lab uses the creatinine cut off plus a negative nitrite test

Of the 10 labs that do not vet requests:
- 1 lab review all clinical details to assess for appropriateness
- 1 lab does not offer UAA as a first line test except for a transport disorder

Q4. What criteria do you use to vet requests?

Q5. Do you have a creatinine cut off below which you would reject a sample?

7 labs with cut offs/caveats said the assay would be run anyway due to a chance an abnormality would be detected.
Q6. Are there any additional screening tests performed routinely on all requests for urine amino acids?

![Pie chart showing the distribution of screening tests](image)

- Dipstick
- Urine Organic Acids
- Nitroprusside
- Sulphitoe
- Reducing Substances
- None

Q7. Are there any other investigations routinely added to requests for urine amino acids?

![Bar chart showing the number of labs using different investigations](image)

Q8. What method of analysis does your laboratory use for urine amino acids?

**Screening:**
- 6 labs run TLC as a screening method
- 1 lab uses ‘Waters MassTrak Amino Acids Kit (derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate followed by UPLC separation & UV detection’
- 10 labs do not perform a screening test prior to quantitation (2 stated they had recently stopped TLC as a screening method)

**Quantitative:**
- 17 labs use Ion Exchange for urine amino acid analysis
- 1 lab refers samples to another lab for quantitation

Q9. What criteria are used to refer samples for quantitation from a screening test?
Q10. Which automated instrument does your lab use for urine amino acid analysis?

Urine amino acids request data
Results from labs which vet requests and those which don’t are presented separately

Q11. How many requests for urine amino acid analysis did you receive between July and December 2012?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=8)</th>
<th>Labs which do not vet requests (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>118</td>
<td>585</td>
</tr>
<tr>
<td>Range</td>
<td>15 - 723</td>
<td>313 - 1000</td>
</tr>
</tbody>
</table>

One lab which vets requests did not include requests for urine cystine.
Q12. If you provide an initial screening test how many samples are referred for quantitative analysis?

Labs which vet requests:
2/8 offer TLC first line and referred 2 and 23% of samples for quantitation respectively.
4/8 labs offer no screening test and performed quantitative analysis on 100% of samples.
2/8 labs did not offer a screening test and referred 13 and 48% of samples for quantitation respectively based on clinical details.

Labs which do not vet requests:
4/10 offer TLC first line and referred 5 – 13 % samples for quantitation.
6/10 offer no screening test and performed quantitative analysis on 100% of samples.

Q13. How many requests for urine amino acid analysis were accompanied by clinical details?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=8)</th>
<th>Labs which do not vet requests (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>93%</td>
<td>87%</td>
</tr>
<tr>
<td>Range</td>
<td>77 - 100%</td>
<td>35 - 90%</td>
</tr>
</tbody>
</table>

Q14. For those requests with clinical details, how may were for the following:

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=6)</th>
<th>Labs which do not vet requests (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic screen/?IMD</td>
<td>65%</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td>32 - 100%</td>
<td>72 - 91%</td>
</tr>
<tr>
<td>Renal stones/cystinuria</td>
<td>10%</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>0 - 29%</td>
<td>5 - 32%</td>
</tr>
<tr>
<td>Renal tubular dysfunction</td>
<td>8%</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>0 - 21%</td>
<td>0 - 8%</td>
</tr>
<tr>
<td>Other</td>
<td>12%</td>
<td>1.20%</td>
</tr>
<tr>
<td></td>
<td>0 - 25%</td>
<td>0 - 6%</td>
</tr>
</tbody>
</table>

Q15. What percentage of urine amino acids requests are from your own Trust?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=8)</th>
<th>Labs which do not vet requests (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>59</td>
<td>25</td>
</tr>
<tr>
<td>Range</td>
<td>13 - 100%</td>
<td>6 - 100%</td>
</tr>
</tbody>
</table>

Q16. How many of the urine amino acid requests also had a urine organic acid requested?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=7)</th>
<th>Labs which do not vet requests (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Range</td>
<td>47 - 100%</td>
<td>49 - 95%</td>
</tr>
</tbody>
</table>

Q17. How many requests also had a plasma sample for amino acids requested?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=7)</th>
<th>Labs which do not vet requests (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>60%</td>
<td>51%</td>
</tr>
<tr>
<td>Range</td>
<td>1 - 67%</td>
<td>22 - 75%</td>
</tr>
</tbody>
</table>
Diagnostic information

**Q18. How many urine amino acids were diagnostic of an IMD other than cystinuria?**

9 labs reported no diagnoses. The other labs reported identification of abnormal UAA in the diagnosis of the following (1 case of each unless otherwise stated):

- OTC deficiency*, CPS1, Citrullinaemia* and 4 other cases of UCD*
- intermittent MSUD*
- NKH
- Homocystinuria* and MTHFR deficiency*
- Hypophosphatasia
- Fanconi of unknown aetiology
- Imminoglycinuria (benign/incidental finding)
- Hartnup
- A sibship with increased taurine excretion due to inveterate Red Bull drinking.
- Generalised amino acid excretion in 20 patients with suspected mitochondrial dysfunction

(*respondents stated that diagnosis was made by other investigations eg plasma AA or OA and that UAA was not the diagnostic test)

**Q19. Were any amino acid results normal in samples from patients subsequently diagnosed with an IMD?**

12 labs reported no diagnoses of IMD in patients with normal UAA, many qualifying this with ‘as far as we know’.
4 labs reported abnormal UAA findings in patients with various organic acid disorders.
2 labs reported homocystinuria with no homocystine detected on UAA analysis (1 CBS deficiency missed by amino acid analyser, 1 MTHFR missed by TLC).

**Q20. To what extent do you agree with the following statement: Urine amino acids should be a first line test for the investigation of IMD?**

![Bar chart showing the extent of agreement]

**Q21. Do you believe that undertaking urinary amino acid analysis adds to other tests performed in the investigation of IMDs?**

![Bar chart showing the frequency of use]

Q22. Under what circumstances should a metabolic laboratory perform urine amino acid analysis?

Q23. Additional comments

Lab A. Forms part of the jigsaw when investigating for IMD. All evidence taken together will provide a stronger base for a proposed diagnosis. Less invasive. May not wish to collect venous samples unless absolutely necessary. Collected in community where centrifuging samples same day may be difficult. MMA observed in organic acids, can easily check for homocystine in urine. Capacity issues if were to run plasma aas on all patients. Diagnosis of IMD relies on many complimentary investigations. A single test can provide a very good idea of the possible defect but rarely is this where the investigation ends. Provided limitations are understood, urine amino acids are a useful tool.

Lab B. Although 80% of samples came with clinical details they are in many instances not very helpful; ‘developmental delay’, ‘behavioural disturbances’ etc.

Lab C. In most circumstances, for the investigation of a suspected IMD, it is not necessary to perform urinary amino acid analysis as a first line test – this analysis can always be added at a later date based on results of the other investigations or the clinical picture.

Lab D. Although clinical details are provided for a large percentage of requests, the information given is often poor (eg ‘global developmental delay’, ‘epilepsy’, ‘?metabolic disorder’)

Lab E. We put a comment on all urine amino acid requests stating that plasma is the preferred sample for the investigation of amino acid disorders.

Lab F. Since the introduction of the revised protocol for metabolic investigations in Oct 2009, the numbers of urine samples with requests for amino acid analysis has dramatically decreased. In 2009 we received 1136 urine samples for ‘metabolic screen’ which had TLC as first line test and quantitation if abnormal or if a cystine level was required. In 2012 we received 360 samples (a decrease of 78%), of which we analysed approximately 50%, based on clinical relevance. From Jan 2013 we are imposing a charge for requests which do not supply clinical information and although early days yet, we have noticed an improvement in compliance. We rarely have been requested to retrieve a sample which was deemed unnecessary for analysis.