

All Wales Clinical Biochemistry Audit Group

Standards for the Laboratory Investigation of Renal Stone Disease

INTRODUCTION

In industrialised communities the incidence of stones is rising; up to 10% of men and 3% of women have identifiable renal stones at some time in their life. Approximately 65 to 70% of individuals undergoing surgery for stones do so for recurrent stones.

A survey of Welsh laboratories, presented at an audit meeting in October 2000, showed significant variations in laboratory strategies for investigating renal stone disease. The following standards have been prepared in the light of these findings and also of published evidence, in consultation with clinical and laboratory colleagues with a special interest in renal stone disease.

STANDARDS

1. Investigation Strategy

- a) Each laboratory should have a written protocol, agreed locally with relevant clinicians, for the initial metabolic investigation of renal stone disease in both adults and children, together with guidelines on further testing depending on the initial results.
- b) The initial blood tests for patients with recurrent stones should include: sodium, potassium, bicarbonate, urea, creatinine, urate, calcium, albumin, phosphate and alkaline phosphatase.
- c) The initial urine tests for patients with recurrent stones should include:
 - calcium, oxalate, citrate, urate and creatinine. In adults a 24 hour collection is preferred; the volume should be measured and results reported as mmol/24hours. In children a random sample can be collected, with results reported as a creatinine ratio.
 - fresh sample (preferably morning) without preservative for qualitative cystine analysis and electrometric measurement of pH. A urine pH <5.5 in the absence of systemic acidosis effectively excludes renal tubular acidosis.
 - random sample with boric acid or other suitable preservative for microscopy/culture.
- d) Recommended further tests should be considered as appropriate:
 - repeat urine collections for calcium, oxalate, urate and citrate (see standard 2 below for recommended number of collections).
 - measurement of urine sodium if there is hypercalciuria.
 - quantitative cystine analysis (24 hour urine in adults, random urine with creatinine measurement in children) if there is a positive cystine screen on analysis of a random urine sample or cystine is detected on stone analysis.
 - screening for partial or complete renal tubular acidosis^{1,2} (appendices 1 and 2) in patients with proven hypercalciuria, in particular those with hypocitraturia or calcium phosphate stones. The furosemide test is recommended for preliminary screening (appendix 1).^{1,2}
 - urine purine analysis for xanthine and dihydroxyadenine (after seeking specialist advice) if xanthinuria is suspected or the patient has radio-lucent stones which do not contain uric acid.
 - urine glycollate and glycerate analysis (after seeking specialist advice). These tests may assist in differentiating primary from secondary causes of hyperoxaluria. Liver biopsy is generally required for definitive diagnosis of the inherited hyperoxalurias.

2. Number of Urine Collections

- a) Single 24 hour urine collections may not be representative. Each laboratory should therefore have an agreed policy on the number of urine samples and collection intervals, with regard to intra-individual variation, for diagnostic and metabolic management purposes.
- b) While recognising the practical difficulties, it is provisionally recommended that at least three 24 hour urine samples, obtained at least 1 week apart, are analysed before concluding that the overall pattern of results is representative (appendix 3).

3. Urine Preservatives³

- a) In recognition of pH solubility/stability characteristics, each laboratory should have policies for preserving urine, in particular 24 hour samples, for calcium, oxalate, citrate, urate and cystine.
- b) Although 24 hour urine collections can be made without special preservatives, pH adjustment and heat treatment (10 minutes at 56°C) is essential to achieve adequate recoveries;⁴ initial collection into a bottle with a suitable preservative is therefore preferred.
- c) It is recommended that 24 hour urine collections should be made into a bottle containing an acid preservative (to achieve a pH of 2.0-2.5) for calcium, oxalate and citrate. The pH should be checked on laboratory receipt and adjusted to 2.0-2.5 if higher.
- d) It is recommended that 24 hour urine collections should be made into a bottle containing an alkali preservative (to achieve a pH of 9.0) for urate. The pH should be checked on laboratory receipt and adjusted to 9.0. Urine collected into alkali is also suitable for cystine analysis.

4. Reference Intervals and Interpretation of Results

- a) Validated reference intervals, age/sex-related as appropriate, should be quoted on reports.
- b) Urine results for children should be reported as analyte to creatinine concentration ratios or per surface area, with appropriate reference intervals^{5,6} (appendix 4) quoted on the report.
- c) Written interpretative information should be available for each assay offered by the laboratory.

5. Renal Calculus Analysis

- a) Clinicians should be actively encouraged to refer renal calculi for analysis, particularly if no previous calculus has been analysed.
- b) Calculi should be analysed using quantitative or semi-quantitative methods. Purely qualitative analysis is not recommended.
- c) Calculi should be analysed for the presence of calcium, magnesium, phosphate, oxalate, urate, carbonate, ammonium ion and cystine.
- d) There should be agreed access to more specialist analyses if required.

6. Quality Control and Assurance

- a) Laboratories should ensure that appropriate internal quality control (IQC) and external quality assessment (EQA) procedures are in place for each assay they perform.
- b) In view of possible "matrix" effects, ideally laboratories should participate in a specific urine EQA scheme for each urine assay performed "in house". As a minimum, serum assays adapted for urine analysis should have been validated for this purpose.
- c) Laboratories should participate in a specific EQA scheme for calculus analysis, if performed "in house".

7. Clinical Management

- a) As appropriate to the resources available and local needs, there should be active involvement of laboratory services in the diagnosis and management of patients with renal stone disease.
- b) It is recommended that the metabolic investigation and management of individuals with renal stones is undertaken via the framework of a specialist clinic, with a defined route of access to expert metabolic advice.
- c) Clinicians should be aware that sustained oral supplementation with Vitamin D at doses exceeding 1000 IU or 25 µg daily may contribute to stone formation.⁷
- d) Clinicians should be aware of strong evidence,⁸ first presented in the early 1990s, against the use of low calcium diets in the clinical management of renal stone disease.

APPENDIX 1 Furosemide Test for Renal Tubular Acidosis^{1,2}

Principle: Acidification of urine normally occurs if an increased amount of sodium is delivered to the distal tubule. It is therefore primarily a test for distal renal tubular acidosis.

Procedure: At 9 am, after an overnight fast (water is permitted), collect baseline samples of urine for pH and venous blood for urea and electrolytes, creatinine and bicarbonate. A basal urine pH below 5.5 precludes the need to proceed further. Give furosemide 40 mg orally and collect urine for pH every 30 minutes for up to 5 hours. [Collect urine into a plain container; immediately aspirate an aliquot into a 5 ml syringe, exclude all air and keep the syringe capped until analysis, within 1 hour.]

Interpretation: Urine pH normally begins to decrease immediately after giving furosemide, but may not reach its nadir for up to 5 hours. A normal response is a decrease in urine pH to less than 5.5. If this does not occur, a further acidification test using ammonium chloride should be arranged.

APPENDIX 2 Ammonium Chloride Load Test of Renal Acidification^{1,2}

Principle: Ammonium chloride induces a metabolic acidosis and should cause acidification of urine.

Procedure: At 9 am, after an overnight fast (water is permitted), collect baseline samples of urine for pH and arterialised capillary blood for pH and base excess. A baseline urine pH below 5.5, or the presence of a metabolic acidosis, precludes the need to proceed further. Give ammonium chloride 0.1 g/Kg body weight orally as a liquid formulation (prepared by pharmacy). It is advisable to give an anti-emetic in advance, e.g. metoclopramide 10 mg i.m. Collect urine for pH and arterialised capillary blood for pH and base excess every hour for up to 5 hours. [Collect urine into a plain container; immediately aspirate an aliquot into a 5 ml syringe, exclude all air and keep the syringe capped until analysis, within 1 hour. Capillary blood samples must be appropriately sealed and analysed promptly.]

Interpretation: Urine pH normally begins to decrease immediately after giving ammonium chloride, but may not reach its nadir for up to 5 hours. A normal response is a decrease in urine pH to less than 5.5, provided an adequate metabolic acidosis has been induced (base deficit > 4 mmol/l).

APPENDIX 3 Evidence for Number of Urine Collections Required

In an unpublished study of 260 patients (Williams CP, Hudson P), components of variance were calculated for consecutive pairs of 24 hour urine collections.⁹ Urine collections with volumes <2 litres or >3 litres were excluded from the analysis. These figures were used to calculate the number of urine samples that would need to be collected to determine an individual's mean urine calcium, phosphate and oxalate excretion $\pm 10\%$, $\pm 20\%$ and $\pm 30\%$.

Test	Intra-individual CV (%)	Inter-individual CV (%)	Analytical CV (%)	No. needed to estimate $\pm 10\%$	No. needed to estimate $\pm 20\%$	No. needed to estimate $\pm 30\%$
Calcium	26.3	40.4	4.8	27	7	3
Phosphate	30.5	24.1	3.3	36	9	4
Oxalate	33.6	22.7	8.0	46	11	5

APPENDIX 4 Reference Intervals for Urine Analyte to Creatinine Ratios^{5,6,10}

Test	Limits	Units	Age range
Calcium/creatinine ratio	≤ 2.00	mmol/mmol Creat	up to 6 months
	≤ 1.40	mmol/mmol Creat	7-18 months
	≤ 0.75	mmol/mmol Creat	≥ 19 months
Citrate/creatinine ratio	≥ 0.100	mmol/mmol Creat	all ages
Oxalate/creatinine ratio	0.015-0.260	mmol/mmol Creat	up to 1 year
	0.011-0.120	mmol/mmol Creat	1-4 years
	0.006-0.150	mmol/mmol Creat	5-11 years
	0.002-0.083	mmol/mmol Creat	≥ 12 years
Urate/creatinine ratio	0.42-1.53	mmol/mmol Creat	up to 2 years
	0.57-1.35	mmol/mmol Creat	2-5 years
	0.39-0.85	mmol/mmol Creat	6-9 years
	0.15-0.67	mmol/mmol Creat	10-17 years
	0.11-0.53	mmol/mmol Creat	≥ 18 years

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VERSION: 1a

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Calendar of Audit Process for Standards for Laboratory Investigation of Renal Stone Disease

Autumn 2000	Survey of Welsh laboratories' strategies for investigating renal stone disease (15/15 laboratories replied) undertaken by Dr.C.Williams and Mr.G.Davies (Maelor Hospital, Wrexham). Findings presented at an All Wales Clinical Biochemistry Audit Group meeting at the Royal Glamorgan Hospital, Llantrisant on 8 th October 2000.
1 st half 2001	Initial draft standards prepared by Dr.C.Williams and Mr.G.Davies and considered at an All Wales Clinical Biochemistry Audit Group committee meeting on 24 th May 2001.
Summer 2001	Draft standards sent for consultation to consultant biochemists and urologists in Wales and Mr.C.Samuell (Consultant Biochemist, UCH, London) to seek their views.
18 th Oct. 2001	Final draft of standards presented at the All Wales Clinical Welsh Biochemistry Audit Group meeting.
22 nd Nov. 2001	Standards ratified at an All Wales Clinical Biochemistry Audit Group committee meeting by Dr.K.Griffiths (chairman).
Spring 2003	Finalised standards issued.
January 2005	Urine calcium/creatinine reference ranges revised.
2006	Proposed date of re-audit and review of standards.