All Wales Clinical Biochemistry Audit Group

Standards for Investigation of Porphyria

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INTRODUCTION

The porphyrias are a group of disorders of haem biosynthesis in which overproduction of haem precursors is associated with characteristic clinical features (Deacon, Whatley et al. 2006). Although porphyrias are uncommon, accurate screening for these disorders, particularly in patients with unexplained abdominal pain or cutaneous photosensitisation, is an important role for clinical biochemistry laboratories.

The key to effective investigation of the porphyrias is the appropriate analysis of correctly chosen samples taken when the patient is symptomatic, taking into account the clinical presentation.

The following standards have been reviewed following re-evaluation of standards previously published in 2004. This review takes into account findings from a second Service User Questionnaire distributed by Wales External Quality Assessment Scheme in 2007 and incorporates published evidence (Elder, Smith et al. 1990; Blake, Poulos et al. 1992; Deacon and Elder 2001) and consultation with experts in the field.

GENERAL STANDARDS

1. Laboratories should encourage appropriate investigations based on clinical findings (particularly distinguishing the cutaneous from the acute porphyrias) by providing clinical-user protocols.

2. The minimum service, required in an acute situation is qualitative analysis for urine porphobilinogen (PBG) within 24 hours (Mustajoki and Nordmann 1993), preferably together with urine total porphyrin (TUP) analysis (see Methodology section). The availability of both tests will provide the ability to make a provisional diagnosis of an acute attack of porphyria.

3. Where cutaneous symptoms are present total urine porphyrin and/or blood analyses should be carried out in a specialist laboratory.

4. Laboratories should review the investigation of porphyrias locally with respect to workload, expertise and economics. Collaboration between neighbouring laboratories permits porphyrin analysis on a regional basis. Alternatively, all porphyrin investigations could be referred to a specialist laboratory that can provide a service in line with Standard 2 above.

5. Laboratories should ensure that they provide an adequate quality of service for “in house” assays:
   a) Wherever possible laboratories should be accredited by CPA (www.cpa-uk.co.uk) or an equivalent accreditation body (CPA standard E6),
   b) Use available validated methods (see Appendix 1),
   c) Offer only assays that have appropriate internal quality control (IQC) and external quality assessment (as per CPA standards F3 for IQC and H5 for EQA),
   d) Have sufficient workload to achieve and maintain evidence-based competence (CPA standard B9.3),
   e) Offer a service for non-urgent tests with a turn around time not exceeding 10 working days,
   f) Report results for quantitative analyses with method specific validated reference ranges.

6. Laboratories should make use of specialist referral centres who are accredited by CPA or an equivalent body (see CPA standard E6 and Appendix 2) for:
   a) Advice (clinical and technical),
   b) The further investigation and identification of the type of porphyria,
   c) Assays which cannot be provided locally,
d) All family studies,
e) Diagnosis during remission.

SAMPLE HANDLING STANDARDS
1. Samples:
   * Urine*: 24 hour collections are unnecessary and inappropriate. A fresh random, preferably early morning sample, protected from light, is preferred for PBG and TUP analysis. Urine samples must NOT be centrifuged before analysis as porphyrins are adsorbed by calcium phosphate and other particulate matter in urine. No preservatives are required.
   * Faeces*: A small portion (5-10g) in a universal container, protected from light
   * Blood*: 5-10ml EDTA anti-coagulated blood, protected from light

2. Storage:
   All samples should be protected as much as possible from light both pre-analytically and throughout the analysis itself. Urine and faecal samples should be stored at or below -20°C unless they are to be assayed on day of receipt. Samples to be sent to a reference laboratory should be dispatched as soon as possible by 1st class post. Whole blood samples should be stored at 4°C and should not be frozen. Separated plasma and erythrocytes can be stored long-term at or below -20°C.

3. The concentration of urine varies widely and can lead to misleading results when porphyrins and their precursors are reported as concentration alone. The reporting of urine porphyrin precursor results as a creatinine ratio is recommended.

4. Care should be taken when interpreting the results from very dilute urine samples (creatinine concentration less than 2mmol/L). Early morning urine samples are preferred.

5. All samples from patients suspected of bullous porphyrias should be treated as high risk due to the strong association of porphyria cutanea tarda with Hepatitis C and HIV (Mansourati, Stone et al. 1999)

METHODOLOGY STANDARDS (see Appendix 1 for available methods)
1. Qualitative porphobilinogen (PBG)
   a) These assays are intended for diagnosis during an acute crisis. They should not be used for diagnosis during remission, for monitoring or for family studies. A positive result in a qualitative PBG assay must be confirmed using a specific and sensitive quantitative assay preferably on the same sample (Deacon and Peters 1998). Particular care should be taken with screening assays in circumstances where PBG levels return to normal or near normal within a few days of onset of an acute attack, e.g. in variegate porphyria or hereditary coproporphyria (Kauppinen and Fraunberg 2002). The potential for false positives or false negatives could have serious clinical consequences.
   b) The Watson-Schwartz screening method described by Pierach et al (Pierach, Cardinal et al. 1977) is rapid, but has been criticised for low sensitivity and specificity (Deacon and Elder 2001). An alternative version that detects excess PBG has been described (Elder, Smith et al. 1990; Deacon and Elder 2001), which performed well (detection limit 12 µmol/L) in a WEQAS trial when fresh reagents were used, but may be misleading if urine samples are very concentrated. Amyl or benzyl alcohols are preferred as the choice of solvent for extraction; chloroform should never be used as it is much less effective and is an important cause of false positive results.
   c) A commercial, semi-quantitative PBG assay is now available. (Trace kit, Alpha Laboratories, Eastleigh, UK)(Deacon and Peters 1998). Its intended purpose is to detect raised PBG concentrations present during an acute attack. The lower detection limit of the assay is 25µmol/L, whereas the upper limit of the reference range is approximately 10µmol/L.
   d) If clinical suspicion of acute porphyria remains high despite a negative qualitative PBG analysis, a full set of samples should be sent to a specialist laboratory for complete analysis.

2. Quantitative porphobilinogen (PBG)
   a) PBG quantification by the Mauzerall and Granick method (Mauzerall and Granick 1956) using anion exchange resin is the most sensitive and specific assay.
   b) A commercial kit is available (Bio-Rad Laboratories, Hemel Hempstead, UK) that includes all technical instructions; it is convenient, but may be expensive for low workloads. A 2ml sample
volume may improve accuracy when measuring low PBG levels (unpublished work performed at Cardiff porphyria service).

3. Urine total porphyrin

a) There are 2 validated methods in use for TUP screening in the UK – Blake (Blake, Poulos et al. 1992) and Deacon (Deacon and Elder 2001).

b) Indicative reference range is $<30\text{nmol/mmoll creatinine}$. However, laboratory specific ranges should be assessed wherever possible.

c) For quantification, a fluorescence method using urine diluted 50-fold in acid (Blake, Poulos et al. 1992) is more accurate and sensitive than spectrophotometry (WEQAS data).

4. Faecal total porphyrin

a) Faecal porphyrin analysis is not a screening test. Its main use is to distinguish between different types of porphyria.

b) Qualitative screening tests using solvent extraction are not recommended; their interpretation is subjective, requiring considerable expertise, with the potential for unreliable results (Deacon 1988).

c) The recommended method is direct spectrophotometry of acidic faecal extract after removal of red fluorescent pigments from dietary chlorophyll with diethyl ether (Lockwood, Poulos et al. 1985). (See appendix for assay details and associated problems).

d) Excess haem in the gastro-intestinal tract, from bleeding or food, converted to protoporphyrin by micro-organisms, is a common cause of an increased faecal porphyrin concentration. A diagnosis of porphyria should never be made by faecal total porphyrin analysis alone (Deacon and Elder 2001).

e) A normal total faecal porphyrin concentration does not exclude erythropoietic protoporphyria. Erythrocyte porphyrin analysis should always be performed if this diagnosis is suspected (Deacon and Elder 2001).

f) Most reference laboratories report faecal porphyrins in terms of dry weight. Where faecal porphyrin is quoted as a wet weight it is essential that an appropriate reference range is used as there is a 4-fold difference between the two methods.

5. Plasma porphyrins

a) Fluorescence emission detection of plasma diluted 10-fold in phosphate buffered saline (Poh-Fitzpatrick 1980) is a simple, rapid qualitative screen for plasma porphyrin.

b) The fluorometric micromethod of Piomelli (Piomelli 1977) can be adapted for plasma porphyrin quantitation. However, it involves an extraction step and will not measure the protein-porphyrin complex present in variegate porphyria. It is however technically exacting and not recommended for use in non-specialist laboratories.

6. Erythrocyte porphyrins

a) Screening methods for erythrocyte porphyrin using solvent extraction or fluorescence microscopy are not recommended; they are unreliable, frequently give false negative or false positive results and are also technically complex (Deacon 1988).

b) The fluorometric micromethod of Piomelli (Piomelli 1977) can be adapted for erythrocyte porphyrin quantitation. It is however technically exacting and not recommended for use in non-specialist laboratories.
7. **Instrumentation**

A fluorescence spectrofluorometer fitted with a red-sensitive photomultiplier is essential for porphyrin analysis.

**RESULTS REPORTING STANDARDS**

1. The laboratory report should state clearly:
   a) The results together with a valid reference range (CPA standard G2.3),
   b) Which type of porphyria has been identified, or which types have been excluded, by the investigations that have been done (CPA standard G2.3); and
   c) Any requirement for further samples, tests and/or information to complete the investigation.

2. If all or some of the investigations have been performed in a specialist laboratory, this should be stated on the final report issued by the local laboratory, together with the interpretative advice supplied by the specialist laboratory (CPA standard G2.4).

3. Clinical advice and interpretive comments should only be provided by authorized personnel with appropriate training (CPA standard G5.3).
Appendix 1 – Validated methods

A. Urine porphyrin

Rationale:
Porphyrins have characteristic absorption spectra that have an intense absorbance peak at around 400nm. Acidification of urine intensifies absorbance, facilitates conversion of porphyrinogens to porphyrins and dissociates zinc-porphyrin chelates. Total urine can therefore be semi-quantitated in acidified urine by spectrophotometry if correction for background absorbance is made.

1. Total urine porphyrin (semi-quantitative) – Deacon Method:

Method
4.0ml of well mixed urine is mixed with 1.0ml concentrated HCL. Undissolved material is removed after acidification by centrifugation. The clear supernatant is transferred to a 1cm cuvette and the absorbance spectrum is recorded between 350 and 450nm blanked against air. The height of the peak above the background is proportional to the porphyrin concentration in the sample. The total porphyrin concentration is: A x 2500nmol/L where A is the peak absorbance, at approximately 400nm, above a baseline drawn between two suitable points (Fig. 1). The factor 2500 is derived from the volume of urine, the volume of acid and a millimolar extinction coefficient of 500, which is approximately that of a 7/1 (mol/mol) mixture of coproporphyrin and uroporphyrin in 2.3M HCL - a porphyrin composition that resembles that of normal urine. Internal quality controls at normal and affected levels should be run with every assay (Recipe ClinChek available from Waters).

Reference range: <30nmol/mmol creatinine

2. Total urine porphyrin (quantitative) – Blake Method:

Method
1.0ml of well mixed urine is mixed with 4.0ml 2.7mol/L HCL. Un-dissolved material is removed by centrifugation. The clear supernatant is transferred to a 1cm cuvette and the absorbance spectrum is recorded between 370 and 440nm blanked against air. The total porphyrin concentration is: A x 14300nmol/L where A is the peak absorbance, at approximately 400nm, above a baseline drawn between two suitable points (Fig. 1). The factor 14300 is derived from the volume of urine, the volume of acid and a millimolar extinction coefficient calculated from molar absorptivities of coproporphyrin and uroporphyrin in 2.7mol/l HCL - a porphyrin composition that resembles that of normal urine. Internal quality controls at normal and affected levels should be run with every assay (Recipe ClinChek available from Waters).

Reference range: <30nmol/mmol creatinine

3. Total urine porphyrin (quantitative) – Fluorimetric method:

Method
20ul well mixed urine is mixed with 1.0ml 0.27mol/l HCL and left in the dark for 5 minutes to allow the conversion of porphyrinogens to their corresponding porphyrins. The diluted urine is then transferred to a 1cm glass cuvette. A coproporphyrin I standard (765nmol/l in a final concentration of 1.5mol/L HCL) is also diluted 1 in 50 with 0.27mol/l HCL. The undiluted standard should be checked for each assay by scanning spectrophotometrically between 350 and 450nm against a 1.5mol/l HCL blank. The absorbance change above the baseline is used as A in the calculation below (See Fig.1). Internal quality controls at normal and affected levels should be run with every assay (Recipe ClinChek available from Waters).
Fluorimeter settings: The fluorimeter must be fitted with a red-sensitive photomultiplier tube and calibrated to give an equal response for coproporphyrin and uroporphyrin. This is carried out by measuring the fluorescence emission maximum of coproporphyrin and uroporphyrin at an excitation of 402nm. A point midway between the emission maxima (usually at approximately 596nm) is used as the emission wavelength. The signal is then measured for excitation at 382, 402 and 422nm.

Concentration of copro standard:

\[
C = \frac{A}{0.489} \times \frac{20}{1020} \times 1000 \text{ nmol/L}
\]

Where \( A \) = maximum absorbance of standard between 350-450 nm.

The total porphyrin concentration:

\[
= \frac{2Su_{402} - Su_{382} - Su_{422}}{2Ss_{402} - Ss_{382} - Ss_{422}} \times C \times 51 \text{ nmol/L}
\]

Where \( Su \) and \( Ss \) are the fluorescent signals (arbitrary units) given by the diluted urine and the standard respectively and \( C \) is the concentration of the standard in nmol/L.

Reference range: <40nmol/mmol creatinine

Notes on TUP measurement (based on unpublished studies)
Urine porphyrin levels decrease rapidly on exposure to light (the half-life is 24hrs). Any urine with a creatinine less than 2.0 mmol/L (the assay sensitivity limit) is unsuitable for analysis. Urines with low creatinine concentrations from children, known acute porphyria patients or patients being treated on an ITU should be interpreted with particular care.

Urine samples must NOT be centrifuged before analysis, as porphyrins are adsorbed by calcium phosphate and other particulate matter in urine.

The “semi-quantitative” assays are classified in this way as a consequence of the inaccuracies resulting from the correction for background absorbance. This is particularly evident when measuring urines with low porphyrin levels and those from patients with other conditions where porphyrin composition is variable.
The concentration of urine varies widely and can lead to misleading results when porphyrins and their precursors are reported as concentration alone; urine creatinine concentrations can vary between <1mmol/l and >40mmol/l depending on hydration. The reporting of urine porphyrin precursor results as a creatinine ratio is recommended.

Urine porphyrin concentrations are increased in symptomatic porphyria cutanea tarda, variegate porphyria, hereditary coproporphyria, congenital erythropoietic porphyria and the very rare 5-aminolevulinate dehydratase porphyria. They are also increased in active acute intermittent porphyria when large concentrations of porphobilinogen are present and non-enzymatic formation of porphobilin and uroporphyrin occurs.

Increased TUP concentrations require further investigation of precursor patterns to distinguish secondary coproporphyrinuria from porphyria. Common causes of secondary coproporphyrinuria include liver dysfunction, alcohol, certain drugs, fever and rarely, lead poisoning.

Normal urine contains mainly coproporphyrin but in latent PCT the proportions of other carboxyl-substituted precursor may be abnormal despite a normal total porphyrin concentration. In patients with skin lesions therefore, where there is a high index of clinical suspicion of porphyria but a normal urine porphyrin excretion, precursor fractionation may be necessary.

### B. Urine porphobilinogen

#### 1. Urine porphobilinogen screen – Watson-Schwartz method

**Rationale:**
Porphobilinogen is reacted with acidified p-dimethylaminobenzaldehyde (modified Ehrlich’s reagent) to form a red condensation product. Saturated sodium acetate is added to intensify this colour. Interfering substances that can also react with Ehrlich’s reagent e.g. urobilinogen are removed by extraction with amyl alcohol leaving porphobilinogen and its red condensation product in the lower, aqueous layer.

**Method**
Modified Ehrlich’s reagent is made by adding 700mg p-dimethylaminobenzaldehyde to 250ml 7M HCL. This reagent is stable in the dark at room temperature for up to 1 month.

1.0ml well mixed urine is added to 1.0ml Ehrlich’s reagent and left to stand for 2 minutes for colour development. 2ml saturated sodium acetate is added and allowed to stand for 5 minutes to neutralise the acid and intensify the colour. 1.0ml amyl alcohol is added; vortex mixed and allowed to stand until the layers have separated. The lower aqueous phase is inspected for pink/red colour. If colour is present in the lower layer the upper organic layer is removed and the aqueous phase re-extracted with 1.0ml aliquots of amyl alcohol until the upper organic layer is colourless. If a pink/red colour persists in the lower aqueous phase this indicates a positive test. A control test to detect false positives due to dyes derived from colouring agents used in foods, medicines, etc. can be performed by substituting Ehrlich’s reagent with 7 M HCL. Internal quality controls at normal and affected levels should be run with every assay (Recipe ClinChek available from Waters).

#### 2. Urine porphobilinogen quantitation – Modified Mauzerall and Granick

**Rationale:**
Porphobilinogen reacts with Ehrlich’s aldehyde reagent (acidified p-dimethylaminobenzaldehyde) to give a red condensation product that has a characteristic absorbance spectrum that has a peak at 553nm and a shoulder at approximately 540nm. Other substances in urine, e.g. urobilinogen, can also react with Ehrlich’s reagent or inhibit the reaction. Accurate quantitation and removal of these interfering substances is carried out using anion exchange chromatography.

**Method**
Modified Ehrlich’s reagent for this assay is made up of 500mg p-dimethylaminobenzaldehyde in 21ml glacial acetic acid and 4ml 70% perchloric acid. This reagent should be made up on the day of assay and is not suitable for longer-term storage.
A suspension of equal parts distilled water to anion exchange resin is made up using AG1-X2 200-400 mesh, anion exchange resin in acetate form (Sigma –Aldrich). This mixture can be stored wet, at room temperature for up to 6 months. Columns are prepared using plastic Pasteur transfer pipettes that have their tops cut off and small balls of cotton wool loosely plugged into the joint between the bulb and the neck. 2ml of well mixed resin slurry is added above the cotton wool filter and washed with 1 column volume of distilled water. The resin should not be allowed to dry out until after elution of porphobilinogen with acetic acid is complete.

1.0ml of well mixed urine is applied to 2ml resin and washed with 1 column volume of distilled water. The PBG is then eluted using 2ml 1M acetic acid followed by 2ml 0.2M acetic acid. The resulting eluate is made up to 5ml with distilled water and mixed thoroughly. 1ml of eluate is mixed with 1ml Ehrlich’s reagent and incubated at room temperature for 15 minutes. A blank consisting of 1ml 1M acetic acid and 1ml Ehrlich’s reagent is also prepared. The solution is transferred to 1ml cuvettes and the absorbance (A) read at 553nm. The concentration of PBG = A x 164umol/L. The factor 164 is derived from the “apparent extinction coefficient” of PBG in modified Ehrlich’s reagent (6.1x 10^4) and the volume of urine and acid used.

Any high results should be checked for interference by checking the complete PBG-Ehrlich’s complex absorbance spectrum between 500 and 600nm. The characteristic spectrum has absorption peaks at 553 and 525nm. The 553nm peak is approximately 1.25 times that at 525nm (Fig 2). The linearity range is 2.5-300umol/l. Any samples with a PBG concentration greater than 300umol/l should be repeated in dilution prior to application on the resin.

Reference range: <10.2umol/l or <1.5umol/mmol Creatinine.

Fig. 2. Characteristic absorbance spectrum of the PBG-Ehrlich’s complex

Notes on quantitative PBG assays
The lower detection limit for the Trace Kit screening test is approximately 25umol/l. Levels of PBG during an acute attack of porphyria are usually many times the upper limit of normal.

Qualitative PBG assays are intended for diagnosis during an acute crisis. They should not be used for diagnosis during remission, for monitoring or for family studies. A positive result in a qualitative PBG assay must be confirmed using a specific and sensitive quantitative assay preferably on the same sample.

Particular care should be taken with screening assays in circumstances where PBG levels return to normal or near normal within a few days of onset of an acute attack, e.g. in variegate porphyria or hereditary coproporphyria. The potential for false positives and false negatives could have serious clinical consequences.

Urine PBG levels decrease rapidly (levels can be halved in 24 hours) on exposure to light. Any urine with a creatinine less than 2.0 mmol/L is unsuitable for quantitative analysis. Urines with low creatinine concentrations from children, known acute porphyria patients or patients being treated on an ITU should be interpreted with particular care.
The assessment of an acute attack in a patient with AIP is often difficult as these patients may have high PBG excretion even in remission. Conversely some patients have abnormal excretion despite never having an acute attack. Although PBG concentration will increase in these individuals during an attack this change is often difficult to detect and careful clinical assessment is required before symptoms are attributed to porphyria.

In a symptomatic AIP patient a normal PBG excretion excludes AIP as a cause of those symptoms.

If clinical suspicion of acute porphyria remains high despite a negative qualitative PBG analysis, a full set of samples should be sent to a specialist laboratory for quantitative PBG and other analyses.

C. Faecal porphyrin

Faecal porphyrin quantitation – Modified Lockwood method

Rationale:
Total faecal porphyrin can be measured by spectrophotometry of an acid extract. Red fluorescent pigments derived from dietary chlorophyll, which also absorb light at 400, must first be removed by extraction with diethyl ether.

Method
Approximately 50mg of faeces is accurately weighed into a glass tube. 1.0ml concentrated HCL is added and vortex mixed until all particles are broken down. 3.0ml diethyl ether is added and mixed to an emulsion before 3.0ml distilled water is added and vortex mixed. The time between addition of concentrated acid and water should be less than 15 minutes to minimise the breakdown of protoporphyrin. The tubes are separated by centrifugation in a spark-free centrifuge. The lower, aqueous layer is transferred to a 1cm cuvette and the absorbance spectrum between 350 and 450nm is recorded. Samples with an absorbance greater than 1.0 Absorbance unit or with high background absorbance are diluted with 2.5M HCL as necessary.

The dry weight of the samples is determined by spreading approximately 200mg wet weight of faeces (+/-10mg) on to a pre-weighed glass slide. The slides are dried using an oven, microwave or rotary evaporator to a constant dry weight of approximately 25% of the wet weight. Standardisation of the drying process is essential and for microwave drying, where conditions depend on batch size, it is necessary to standardise for one batch size which is not altered. After cooling the slides are re-weighed to obtain the dry weight.

For a peak at approximately 400nm the total porphyrin (nmol/g dry weight) is given by:

\[
= \frac{A \times D \times 4.5 \times ww}{0.3285 \times t \times dw}
\]

Where A is the corrected absorbance between 350 and 450nm (see fig. 1), 0.33 is the average micromolar extinction coefficient for a mixture of protoporphyrin and coproporphyrin (3/1; mol/mol) in 2.5M HCL, 4.5 is the volume of the aqueous phase in millilitres, D is the dilution factor of extract (usually 1), t is the weight of faecal sample taken for analysis, ww is the wet weight of faecal samples used in the dry weight determination and dw is the dry weight of faecal sample used in the dry weight determination. All weights are in grams.

There is no commercially available IQC material available for faecal porphyrin measurement. Controls can be prepared by homogenising pools of faeces with normal and abnormal porphyrin levels, dividing into approximately 0.5g aliquots and storing at -20°C for up to 1 year.

Reference range: <200nmol/g (dry weight) or 50nmol/g (wet weight)

Notes on faecal porphyrin quantitation
Porphyrins are rapidly degraded by peroxides that can accumulate in diethyl ether. Analar grade diethyl ether contains <0.000015% ether peroxides and is suitable for use. It should be stored in the dark and discarded after 2 months.
Qualitative screening methods that depend on solvent extraction are considered unreliable and should no longer be used.

Faecal porphyrin excretion is increased in active porphyria cutanea tarda, variegate porphyria, hereditary coproporphyria, congenital erythropoietic porphyria, some patients with erythropoietic protoporphyria, and may be moderately increased in acute intermittent porphyria.

Secondary causes of increased faecal porphyrin concentration include increased haem in the gut from occult bleeding, bleeding gums or dietary sources.

Faecal porphyrin fractionation should be used to distinguish true porphyria from secondary causes of increased excretion. It can also differentiate between PCT, VP, HCP and CEP. It can also distinguish between PCT and patients with the similar bullous skin lesions seen in some patients with chronic renal failure and in drug-induced and other pseudoporphyrias.

Faecal porphyrin measurement is not suitable for the diagnosis of EPP as at least 40% of these patients have normal concentrations and increased levels as a result of secondary conditions may lead to false positive results.
Appendix 2 – Porphyria referral laboratories

- Cardiff Porphyria Service
  Department of Medical Biochemistry and Immunology
  University Hospital of Wales
  Heath Park
  Cardiff
  CF14 4XW
  Tel: 029 20743565 (Lab)
  029 20746588 (Secretary to Dr. Badminton)
  Fax: 029 20748383

- Department of Biochemistry
  King’s College Hospital
  Denmark Hill
  London
  SE5 9RS
  Tel: 02032993856 (Dr. J. Marsden)

- Department of Clinical Biochemistry
  Salford Hospital
  Stott Lane
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  Fax: 0161 7887443

- Department of Clinical Biochemistry
  United Leeds Teaching Hospitals
  Britannia House
  Britannia Road
  Morley
  LS27 0DQ
  Tel: 0113 3927850 (Dr. K. Allen)
  Fax: 0113 3927815

References


[www.cpa-uk.co.uk](http://www.cpa-uk.co.uk) Standards for the Medical Laboratory, Clinical Pathology Accreditation (UK) Ltd.