New Instructions for Sending Samples for Diagnosis and Monitoring of Inborn Errors of Bile Acid Synthesis (IEBAS)

We are introducing an improved method for diagnosis and monitoring inborn errors of bile acid synthesis based on dried blood spots (DBS) instead of urine samples for the following reasons:

1. It can be difficult to get a urine sample from an infant
2. We have asked that, if urine samples are not going to get to us within 24 hours they had to be frozen and sent on card ice. This was difficult for some hospitals. Dried blood spots are easy to obtain and can be sent in the post; the bile acids that we measure for diagnosis of IEBAS are stable for at least 4 weeks
3. The urine method detects all IEBAS presenting with cholestatic liver disease / fat-soluble vitamin malabsorption in the first 18 months of life. In the case of inborn errors of bile acid synthesis that present beyond the age of 18 months e.g. an adult with neurological disease due to α-methyl-CoA racemase deficiency, we know a urine cholanoid spectrum of urine bile acids and bile alcohols is not sensitive enough to pick up the abnormal bile acids (C27 bile acids [cholestanoic acids] in AMACR deficiency) but we can detect AMACR deficiency and other late-presenting bile acid synthesis disorders by analysis of a dried plasma or blood spot.
4. For monitoring the treatment of the two disorders for which cholic acid is the NICE-approved treatment (3β-hydroxy-Δ5-C27-steroid dehydrogenase deficiency and Δ4-3-oxosteroid 5β-reductase deficiency), we have monitored a few patients using a quantitative analysis of bile acids in dried blood spots and the results suggested that this method could give a better indication of whether the dose of cholic acid is correct for the patient.

So, we are proposing to switch to a quantitative analysis of blood spots for all diagnosis and monitoring of IEBAS but for an initial period of 6 months, we will run urine and DBS samples in parallel. The charge will be unchanged from that of the urine spectrum (£150).

We are intending to run samples once a week and will try and achieve a turnaround time of less than 4 weeks (at least for the urine result) but sometimes this may be impacted by factors outside our control. Where results are required more urgently, please contact us to discuss.

Enquiries about sample requirements can be addressed to y.khalil@ucl.ac.uk or r.hirachan@ucl.ac.uk

Enquiries about results should be directed to peter.clayton2@nhs.net This is a secure email address so please include patient identifiers to facilitate retrieving the result. Please do not include patient identifiers in e-mails to y.khalil@ucl.ac.uk or r.hirachan@ucl.ac.uk as these e-mail addresses are not secure.

Sample Requirements (send all 3 types of sample, please)

Liquid urine sample

- Minimum volume 0.5ml
- Plain leakproof container (no preservative)
- Does not need to be frozen but should preferably arrive within 5 days of sample collection.

Urine Spots

- Spot 20 microlitres onto the centre of the circle of a newborn screening card* x 4 (4 separate spots)
  *Guthrie card (Whatman 903 paper)
- Where possible, try not to use very dilute urine, as this will make it difficult to see the urine spots for analysis purposes.
- Label the card "Urine spots".
- Dry overnight at room temperature.
- Put the card inside a glassine paper and then in an envelope.

Dried Blood Spots*

- Proceed as for other blood spot test on infants.
- Use a Guthrie newborn screening card (Whatman 903 paper)
- Obtain blood at the cotside by a heel prick**.
- Fill the circle completely with a single drop of blood; do not double-spot.
- Fill 4 circles, if possible, please
  **For older children and adults use a finger prick
- Dry overnight at room temperature.
- Put the card inside a glassine paper and then in an envelope.

*If the only sample available from blood is a stored serum/ plasma sample, spot 10 microlitres of plasma into each circle on the Guthrie card and make sure you label the card "Plasma spots".

Address for all 3 samples:

Diagnostic tests (lab of Philippa Mills and Peter Clayton)

Inborn Errors of Metabolism

Genetics and Genomic Medicine

UCL Great Ormond Street Institute of Child Health

30 Guilford Street
London WC1N 1EN
(UK)

Please make sure that samples will not arrive late on Friday or at the weekend or on a Bank Holiday.

Request Form for Diagnosis and Monitoring of Inborn Errors of Bile Acid Synthesis (IEBAS)

Surname:

First name(s):

Date of Birth:

NHS Number:

Date of samples:

Is this individual known to have an inborn error of bile acid analysis? If so, which?:

Is this individual suspected to have an inborn error of bile acid synthesis on the basis of DNA analysis:

Gene:

Variants detected:

Bilirubin (conjugated):

ALT:

AST:

GGT:

Any neurological / developmental problems?

Is this individual on any bile acid treatment*?:

Cholic acid: Y/N

Dose (mg/kg/d)

Ursodeoxycholic acid: Y/N

Dose (mg/kg/d):

Chenodeoxycholic acid: Y/N

Dose (mg/kg/d)

*Please note that for an initial diagnostic sample it is preferable that the individual is not on any bile acid therapy

Email address(es) for results (please include a generic address for the lab as well as an individual address; both must be nhs.net addresses):

Billing address:

For the qualitative result on DBS and the qualitative result on urine we will charge the same as we have traditionally charged for the qualitative analysis on urine alone (£150).

Format for Reporting the Results

- **Urine Cholanoid (Bile Acid and Bile Alcohol) Spectrum**

New Patient

As previously this will be reported as one of the following interpretations:

- No evidence of an inborn error of bile acid synthesis.
- Taurotetrahydroxycholestanoic acid(s) present suggesting the diagnosis of a peroxisomal disorder.
- Taurotetrahydroxycholestenoic acid(s) present suggesting the diagnosis of a disorder of the peroxisomal bifunctional protein.
- Sulphated dihydroxy- and trihydroxy-cholenoic acids present as major peaks indicating a probable diagnosis of 3beta-hydroxy-delta5-c27-steroid dehydrogenase deficiency.
- Cholestanepentol glucuronides present in large amounts indicating a probable diagnosis of cerebrotendinous xanthomatosis (CTX).
- Hydroxy- and dihydroxy-3-oxo-4-cholenoic acids present as major urinary bile acids. This has been attributed in some patients to a deficiency of delta4-3-oxosteroid 5beta-reductase.
- Monohydroxy-5-cholenoic acids present as major peaks suggesting a diagnosis of oxysterol 7alpha-hydroxylase deficiency.
- Major urinary bile acids are non-amidated suggesting an amidation defect.
- No evidence of an inborn error of bile acid synthesis. Normal bile acids present in larger amounts than normal indicating cholestasis.

Patient with 3β-hydroxy-Δ5-C27 steroid dehydrogenase deficiency or Δ4-3-oxosteroid 5β-reductase deficiency on treatment with cholic acid

The report will be one of the following:

- The excretion of unsaturated bile acids is at a low level and saturated bile acids from the treatment are detectable suggesting a good response to treatment.
- High levels of excretion of unsaturated bile acids with saturated bile acids from treatment not detectable suggesting non-compliance or dose of cholic acid sub-optimal.

The urine report will be issued first in some cases with a report saying results on the dried blood spots to follow.

- **Blood Spot Quantitative Bile Acid Analysis**

As with the urine the blood spot / plasma analysis will be reported as an interpretation as follows:

New Patient

- No evidence of an inborn error of bile acid synthesis
- Trihydroxycholestanoic acid(s) (THCA) and /or dihydroxycholestanoic acid(s) (DHCA) above normal range suggesting the diagnosis of a peroxisomal disorder.
- Dihydroxy- and trihydroxy-cholenoic acids above normal range indicating a probable diagnosis of 3beta-hydroxy-delta5-c27-steroid dehydrogenase deficiency.
- Cholestanetetrol glucuronides above normal range indicating a probable diagnosis of cerebrotendinous xanthomatosis (CTX).
- Hydroxy- and dihydroxy-3-oxo-4-cholenoic acids above the normal range. This has been attributed in some patients to a deficiency of delta4-3-oxosteroid 5beta-reductase but can also occur with severe hepatocyte damage.
- 3beta-hydroxy-5-cholenoic acids and 3beta-hydroxy-5-cholestenoic acids above normal range suggesting a diagnosis of oxysterol 7alpha-hydroxylase deficiency.
- The major bile acids in blood/plasma are non-amidated suggesting an amidation defect.
- No evidence of an inborn error of bile acid synthesis. Normal bile acids present in larger amounts than normal indicating cholestasis.

Patient with 3β-hydroxy-Δ5-C27 steroid dehydrogenase deficiency or Δ4-3-oxosteroid 5β-reductase deficiency on treatment with cholic acid

The report will be one of the following:

- The blood levels of unsaturated bile acids are low level and saturated bile acids from the treatment are clearly detectable suggesting a good response to treatment.
- High levels of excretion of unsaturated bile acids with saturated bile acids from treatment not detectable or at a low-level suggesting non-compliance or dose of cholic acid sub-optimal.
- The level of saturated bile acids (from the treatment) is higher than desirable; consider a reduction of dose of cholic acid.
- A quantitative result will also be sent, taking the form of one or two of the following (usually one report of the abnormal bile acids and one of the normal bile acids.

Requestor

Address Line 1

Address Line 2

Address Line 3

Address Line 4

Address Line 5

Date of report

Name: **Lab number:**

Date of birth: **ICH number:**

Date of sample:

Bile acids associated with 3β-hydroxy-Δ5-C27-steroid dehydrogenase deficiency

Bile acid	Patient result	Arbitrary Reference Range (Arbitrary units)
3β-sulfooxy-7α-hydroxy-5-cholenoic acid		
3β-sulfooxy-7α,12α-dihydroxy-5-cholenoic acid		
3β-sulfooxy-7α-hydroxy-5-cholenoyl glycine		
3β-sulfooxy-7α,12α-dihydroxy-5-cholenoyl glycine		
3β,7α-dihydroxy-5-cholenoyl glycine		
3β,7α,12α-trihydroxy-5-cholenoyl glycine		
3β,7α-dihydroxy-5-cholenoyl taurine		
3β,7α,12α-trihydroxy-5-cholenoyl taurine		

Bile acids associated with Δ4-3-oxosteroid-5β-reductase deficiency

Bile acid	Patient result	Arbitrary Reference Range (Arbitrary units)
7α-hydroxy-3-oxo-4-cholenoyl glycine		
7α,12α-dihydroxy-3-oxo-4-cholenoyl glycine		
7α-hydroxy-3-oxo-4-cholenoyl taurine		
7α,12α-dihydroxy-3-oxo-4-cholenoyl taurine		

Bile acids associated with Cerebrotendinous xanthomatosis

Bile acid	Patient result	Arbitrary Reference Range (Arbitrary units)
5β-cholestane-tetrol glucuronides[1]		
5β-cholestane-pentol glucuronide		

[1]5β-cholestane-3α,7α,12α,25-tetrol glucuronide is the major peak.

Bile acids associated with a Peroxisomal disorder

Bile acid	Patient result	Reference Range (μM)	Arbitrary Reference Range (Arbitrary units)
Trihydroxycholestanoic acid			
Glyco-trihydroxycholestanoic acid			
Tauro-trihydroxycholestanoic acid			
Dihydroxycholestanoic acid			
Glyco-dihydroxycholestanoic acid			
Tauro-dihydroxycholestanoic acid			
Glyco-tetrahydroxycholestanoic acid			
Tauro-tetrahydroxycholestanoic acid			
Tauro-tetrahydroxycholestenoic acid			

Bile acids associated with Oxysterol-7α-hydroxylase deficiency

Bile acid	Patient result	Arbitrary Reference Range (Arbitrary units)
3β-sulfooxy-5-cholenoic acid		
3β-hydroxy-5-cholenoyl taurine		
3β-sulfooxy-5-cholenoyl glycine		
3β-hydroxy-5-cholestenoic acid		

Bile acids associated with an Amidation defect

Bile acid	Patient result	Reference Range (μM)	Arbitrary Reference Range (Arbitrary units)
Cholate			
Cholic acid glucuronide			
Chenodeoxycholic acid glucuronide			

Bile acids associated with Cholestasis

Bile acid	Patient result	Reference Range (µM)	Arbitrary Reference Range (Arbitrary units)
Glycocholate			
Glycodihydroxycholanoates[2]			
Tetrahydroxy-cholanoyl glycine			
Taurocholate			
Taurodihydroxycholanoates[3]			
Tetrahydroxy-cholanoyl taurine			

[2]Glycochenodeoxycholate and Glycodeoxycholate not separated.

[3]Taurochenodeoxycholate and Taurodeoxycholate not separated.

Bile acids associated with Ursodeoxycholic acid treatment

Bile acid	Patient result	Arbitrary Reference Range (Arbitrary units)
Ursodeoxycholic acid		
Glycoursodeoxycholic acid		
Tauroursodeoxycholic acid		

Conclusion

Professor Peter T Clayton MD, FRCP, FRCPCH

Genetics and Genomic Medicine, UCL Great Ormond Street Institute of Child Health

Although fully validated by our laboratory, this test is not UKAS accredited

Enquiries about results that include patient identifiers should be sent to peter.clayton2@nhs.net or philippa.mills@nhs.net . General enquiries about sample requirements or when testing should be undertaken should be addressed to peter.clayton@ucl.ac.uk or p.mills@ucl.ac.uk or y.khalil@ucl.ac.uk