September 2016

pathology@viapath

Message from the Editor

Welcome to our first edition of pathology@viapath, updating you with what's happening at Viapath. Hopefully you'll find the contents useful, informative and maybe even entertaining!

As you may know, Viapath is a pathology business, majority owned by the NHS and provides 20 million tests a year to nearly 1,000 different healthcare organisations, mostly within the NHS. Viapath shares NHS values and each year reinvests its surpluses into pathology innovation, staff development and quality services.

If you have any comments or suggestions for future editions of this newsletter, please get in touch!

Inside this issue:

Context and Application of Targeted Genetic Testing

A newly published book chapter by Joo Wook Ahn & Caroline Ogilvie, members of the Genetics Laboratories, on the Context and Applications of Targeted Genetic Testing, with Emphasis on Copy Number Variants.

Abstract

There has been a huge acceleration in our technical ability to detect variation in the human genome in recent years, and there has been a corresponding effort in clinical diagnostic laboratories to take advantage of this progress for the benefit of patients. There has, however, not been an equivalent increase in our understanding of human genetics and disease, not for lack of effort but due to the far greater complexity of understanding variation than the difficulties of detecting it. This chapter describes how software tools can be used to target clinical genetic diagnostic testing in order to exploit technical and scientific advances both efficiently and cost-effectively, while maximizing clinical utility.

Adv Clin Chem. 2016;75:33-51. doi: 10.1016/bs.acc.2016.03.004. Epub 2016 Apr 20.
http://www.ncbi.nlm.nih.gov/pubmed/27346615

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Zika Virus: News and Views

Zika is a mosquito-borne infection caused by Zika virus (ZIKV), a member of the genus flavivirus and family Flaviviridae. It was first isolated from a monkey in the Zika forest in Uganda in 1947.

On 1 February 2016, it was declared that the cluster of microcephaly cases and other neurological disorders reported in Brazil, following a similar cluster in French Polynesia in 2014, constituted a Public Health Emergency of International Concern (PHEIC).

As surveillance for Zika virus infection improves, further cases are expected to be reported in affected regions (Pacific islands and countries of South America like Brazil) and previously unaffected countries, particularly in south and central America and the Caribbean, where the Aedes mosquito vector is present.

Zika Cases Diagnosed in the UK

ZIKV does not occur naturally in the UK. However, as of 21 July 2016, a total of 50 cases have been diagnosed in UK travellers since 2015.

Transmission

Zika virus is most commonly transmitted by the bite of an infected female Aedes mosquito, mainly Aedes aegypti. The Aedes aegypti mosquito is not present in the UK as the UK temperature is not consistently high enough for it to breed. After an infected mosquito bites a human, the first symptoms of Zika can develop in 3 to 12 days. Other means of transmission are from mother to foetus via the placenta and through sexual transmission.

Symptoms

The majority of people infected with Zika virus are asymptomatic. For those with symptoms, Zika virus causes a mild, short-lived (2 to 7 days) illness. Signs and symptoms suggestive of Zika virus infection may include a combination of the following:

- rash
- itching/pruritus
- fever
- headache
- arthralgia/arthritis
- myalgia
- conjunctivitis
- lower back pain
- retro-orbital pain

The symptoms of Zika are similar to dengue (caused by a related flavivirus) or chikungunya (an alphavirus), which are often co-circulating in areas where Zika virus is present. Laboratory testing is essential for the correct diagnosis.

Diagnosis

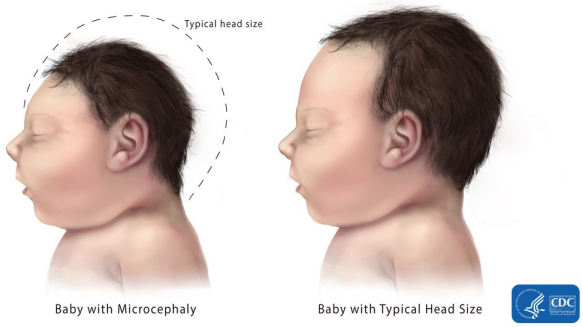
- Zika virus RNA is only detectable in blood for a few days (5 days) after symptoms begin, whilst Zika antibodies often appear within a week of symptom onset
- In urine it is detected for longer, 10-14 days
- In semen it can be detected for as long as 62 days

Testing only occurs in patients who exhibit signs of infection either whilst in a country with active Zika virus transmission or within 2 weeks of travel

* Pregnant woman with current symptoms: serum, EDTA blood, urine
* Man with current symptoms whose partner is pregnant: serum, EDTA blood, urine
* All other male and female patients with current symptoms: serum, EDTA blood, urine

Men or non-pregnant women who have never experienced symptoms suggestive of Zika virus infection do not require testing.

For suspected cases of Zika virus infection, send samples to Public Health England's Rare and Imported Pathogens Laboratory (RIPL), via the local diagnostic laboratory.



Latest Guidance:
Information for primary care and clinicians has been jointly developed by PHE, Royal College of General Practitioners and the British Medical Association:
Zika virus infection: guidance for primary care

For the latest government advice:
https://www.gov.uk/guidance/zika-virus-travel-advice https://www.gov.uk/guidance/zika-virus sex
https://www.gov.uk/guidance/zika-virus pregnancy
Researched and written by Simantee Guha, Consultant Microbiologist, Bedford Hospital

Listen to Pathology Podcasts from Viapath's Future Leaders in Innovation Group

Click the icon to listen to the podcast

Episode One : Highlights from Viapath's 4th Innovation Symposium

* Dr Dominic Harrington, Viapath's Scientific Director, discusses the driving force behind the Innovation Academy.
* Vivienne Parry OBE talks about Genomics England's 100k Genome Project
* Dr Foco Zandbergen discusses the need for more education on vitamin K and its future testing.

Episode Three: Improving the diagnosis of inherited metabolic diseases and the pharmacogenomics of purine and pyrimidine drug analogues; by Dr Tony Marinaki.

Episode Five: It's Diagnosis not Diagnostics!

* Andy Brogan discusses being Practice Lead for Health and Social Care, Vanguard Consulting.
* Dr Jignesh Patel discusses anticoagulant therapy, and how he will be optimising DOACs in the patient population.

'The trend in genomic analysis is very much in the direction of sequencing... particularly next generation sequencing' Professor Graham Taylor, Episode Ten

Episode Nine: Viapath; past, present and future. A discussion with Richard Jones, CEO of Viapath.

Episode Ten: Acquiring, managing and sharing genomic data for clinical benefit. A discussion with Professor Graham Taylor, head of Genomics at Viapath.

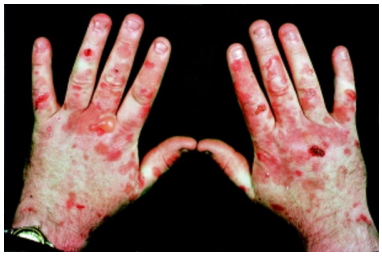
Viapath's Porphyria Laboratory Now Offers An Urgent Urine Porphobilinogen (PBG) Service

Viapath's Porphyria Laboratory, headed by Dr Joanne Marsden, is one of only two Supraregional Assay Service (SAS) designated laboratories in the UK offering diagnosis of rare porphyria disorders.

The porphyrias are a group of mainly inherited diseases of the haem synthesis pathway. The porphyrias are classified into acute and cutaneous depending on their presenting symptoms. There are eight different porphyrias and they can present with symptoms such as severe abdominal pain, blisters or photosensitivity. Porphyria are diagnosed using urine, blood and stool specimens and techniques such as ion exchange chromatography, spectrophotometry and fluorimetry and more recently, genetic analysis.

Patients with an acute porphyria can have life threatening attacks that usually require admission to hospital and specific treatment. The biochemical hallmark of an acute porphyria attack is the increase in urine excretion of urine porphobilinogen (PBG) that can increase significantly during an attack. Recent guidelines from the British and Irish Porphyria Network (BIPNET) have recommended that urine is analysed promptly for quantitative PBG concentration (Ann Clin Biochem 2016, in press).

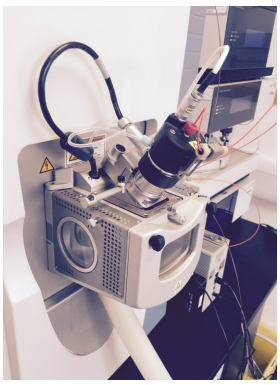
Only a few laboratories are able to perform the analysis due to restraints in technology and staff training. The Porphyria Laboratory is now able to offer an urgent weekday service for PBG analysis (Monday to Friday during working hours) to internal and external customers. Results will be reported by telephone on the same day of analysis. The method is based on ion-exchange chromatography and a colour reaction with Ehrlich's reagent. It is recommended that a positive result is followed up by providing blood and stool samples to confirm the type of porphyria. The patient is also referred to the Porphyria Clinic for advice on management of the disease. Porphyria genetics are also available and performed in Viapath's Molecular Department for inheritance studies.



Please contact the Porphyria Laboratory on 020 3299 3856 to discuss.

Focus on: The Drugs of Abuse Testing Laboratory

Modernising urine drugs of abuse testing at Viapath



Testing for drugs of abuse is an ever-evolving field. In the Toxicology Laboratory, the process used to test for drugs of abuse in urine changed in November 2015. Samples are no longer 'screened' a range of immunoassays and then some drugs 'confirmed'. Instead, all urines are now analysed directly by high-resolution mass spectrometry for a range of *specific* drugs and metabolites. As well as a faster overall turnaround, this approach also enables us to look for additional compounds such as dihydrocodeine on all samples as part of the Standard Urine Drug Screen. There are also additions to the drug testing repertoire, such as ketamine, mephedrone and tramadol, as part of the new 'Premium Urine Drugs Screen'. Other new tests, including

Identifying sample dilution and/or substitution

All urine samples analysed in the laboratory have creatinine measured as a check on sample integrity. Samples with a creatinine below 2.0 mmol/L are reported with a comment highlighting that the sample is 'dilute' and those with a creatinine concentration below 0.5 mmol/L with a comment stating that the sample is 'extremely dilute'. A non-detectable creatinine might suggest the sample has been substituted with, for instance, weak tea or fruit juice!

Which drugs are measured by mass spectrometry?

High-resolution mass spectrometry is used to test for the compounds listed below, which are reported individually. No further confirmation is required. Cut-off concentrations used are based on the European Workplace Drug Testing Guidelines.

- Opiates are reported separately as 'positive' for samples with a total drug + metabolite concentration greater than **300 ng/mL** for the following drugs:
  - Morphine and metabolite (morphine-3-glucuronide)
  - Codeine and metabolite (codeine glucuronide)
  - Dihydrocodeine and metabolite (dihydrocodeine glucuronide)
- 6-Acetylmorphine (sometimes called 6-monoacetylmorphine, a specific heroin (diamorphine) metabolite), is reported as positive for samples with a concentration greater than **10 ng/mL**
- Amfetamines are reported separately as 'positive' for samples with concentrations greater than **200 ng/mL** for the following drugs:
  - Amfetamine ('Speed')
  - Metamfetamine ('Meth', 'Crystal meth')
  - MDMA ('Ecstasy')
- Cocaine (measured as the metabolite benzoylecgonine) is reported as 'positive' for samples with a benzoylecgonine concentration greater than **150 ng/mL**
- Methadone and its major methadone metabolite, EDDP, are reported separately as 'positive' at concentrations greater than **250 ng/mL**

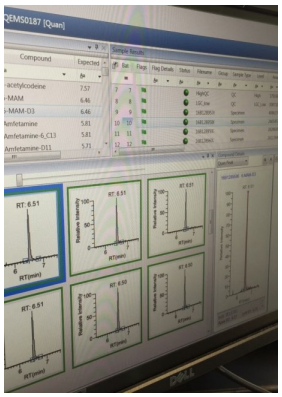
The additional drugs and metabolites reported when a Premium Urine Drug Screen is requested are listed below:

- Buprenorphine is reported as 'positive' for samples with concentrations greater than **5 ng/mL**
  - The sum of the major buprenorphine metabolites (norbuprenorphine, buprenorphine glucuronide and norbuprenorphine glucuronide) is also reported as 'positive' for samples with a total concentration greater than **5 ng/mL**
- Ketamine is reported as 'positive' for samples if the total concentration of ketamine and the major metabolite, norketamine, is greater than **50 ng/mL**
- Mephedrone is reported as 'positive' for samples with concentrations greater than **200 ng/mL**

- Tramadol is reported as 'positive' for samples if the total concentration of tramadol and its major metabolites, *N*-desmethyltramadol and *O*-desmethyltramadol, is greater than **200 ng/mL**

Which other analytes are measured?

All urine samples are routinely analysed by immunoassay for cannabis (tetrahydrocannabinol: THC) and cannabis metabolites, and for benzodiazepines. Barbiturates are not screened for routinely because they are rarely encountered nowadays but can still be tested for on request for individual samples. Urine or plasma alcohol (ethanol) is measured by an enzymatic assay and, although not measured routinely for every sample, can also be requested. If requesting an alcohol measurement on urine, a non-preserved sample is suitable. However, if plasma alcohol is required, the sample should be collected into a fluoride oxalate blood collection tube.



- Benzodiazepines are reported as 'positive' for samples with a total concentration greater than **300 ng/mL**
- Cannabis is reported as 'positive' for samples with a total concentration greater than **50 ng/mL**
- The concentration of urine/plasma alcohol is reported, as opposed to a 'positive' or 'negative' report. The limit of detection for the test is **10 mg/dL**

The importance of testing for metabolites

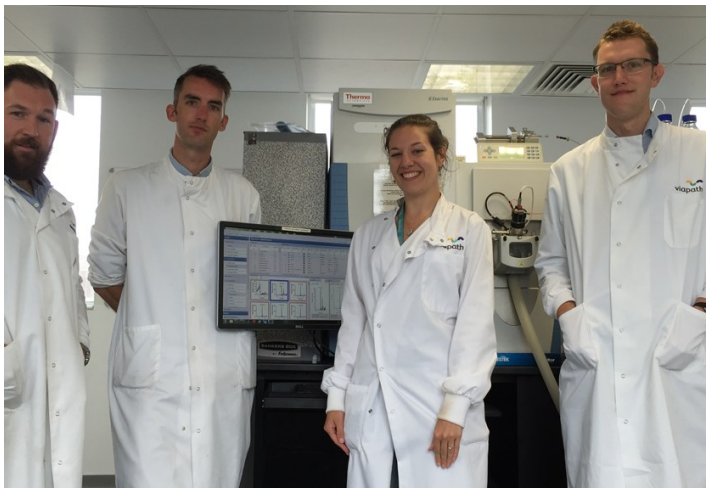
The methadone metabolite, EDDP, and metabolites of buprenorphine are measured separately to help identify possible sample adulteration (i.e. the addition of parent drug to the sample). A 'positive' result from an immunoassay or dipstick for replacement medications, such as methadone or buprenorphine (as Subutex or Suboxone), can arise from adulterated urine samples since the metabolites are not measured separately. For buprenorphine taken sublingually, clients spitting out partially-absorbed tablets concealed under the tongue into urine pots to produce falsely positive results could also be possible. It is for these reasons that the laboratory report includes separate results for methadone and EDDP and buprenorphine and buprenorphine metabolites.

Identifying substances

Our high-resolution mass spectrometer can also be used to help identify unknown tablets, powders and capsules. We have previously analysed smoking products to identify synthetic cannabinoids, cigarette butts and contents of syringes. For such requests, please contact the laboratory prior to sending any samples.

Further questions or queries?

Further information on the Urine Drugs of Abuse Testing Service is available from the laboratory or from the Viapath website. Request forms and the User Information Leaflet are also available to print and download for clinic staff.
Please contact the laboratory with any specific requests on cases for drugs not currently listed we may be able to help!
Web: www.viapath.co.uk/departments-and-laboratories/drugs-of-abuse-laboratory-at-kings
Tel: 0203 299 5881



The 'Drugs of Abuse' team and high-resolution mass spectrometer in use for urine testing at Kings College Hospital.

Viapath llp
Francis House
9 Kings Head Yard
London
SE1 1NA

Phone: 020 7188 2500
E-mail: newsletter@viapath.co.uk

Viapath is a unique partnership of clinical, scientific and operational expertise, with a mission to transform pathology services in the UK. Our organisation is built on scientific expertise, providing a service that helps clinicians create better outcomes for their patients every day.

We are continually focused on innovation, finding new and better ways to manage the logistics of high-volume pathology testing as well as specialist reference testing. We always strive to improve capabilities to better meet our customers' needs.

We are a scientific organisation with a clinical purpose.

www.viapath.co.uk

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