Public Health England

Public Health England
Rare & Imported Pathogens
Porton Down
Salisbury
Wiltshire, SP4 0JG

Tel +44 (0)1980 612774
Fax +44 (0)1980 612695

24th January 2018

Dear Laboratory Director and Quality Manager

Re: Changes to the RIPL Lyme Disease service

The Rare & Imported Pathogens Laboratory (RIPL) will be making a number of changes to the Lyme disease testing service to improve the quality and turn-around times of the service from the beginning of February 2018. The changes are detailed below, but in summary:

1. The C6 screening test is unchanged and will be performed on all serum samples.

2. The serum Lyme blot using the ViraMed ViraStripe system will be replaced by an updated version using the same antigens in a new array format. This version, the ViraMed ViraChip offers higher precision and doubles the throughput. The sample requirements and costs per test are unchanged.

3. For CSF samples, a contemporaneous serum sample MUST be submitted with the CSF sample. Assays will be run in parallel with serum on the ViraChip, allowing direct comparison between antibody levels in serum which has been diluted to allow a direct comparison between the serum and CSF assays allowing for the inherent difference s in immunoglobulin and protein levels. This enables an accurate assessment of intrathecal antibody production to be made when used in conjunction with the measured intensity of antibodies to Borrelia detected in the CSF.

4. To achieve this, we will be testing the albumin and immunoglobulin levels in the serum and CSF samples prior to running the parallel assays. If these levels are provided by the source laboratory, the costs will remain the same; otherwise we will arrange for the levels to be measured and a small charge (~£27) will be added to the fee. Turn-around times for CSF will be typically 3 to 5 days longer than for serum because of the limited numbers and the multiple steps involved in the analysis. An additional 7 days should be allowed if protein and immunoglobulin levels are not provided with sample details.

5. A new PCR test for certain samples such as tissue and CSF will be introduced at the same time, covering a wider range of Borrelia species rather than just the *B. burgdorferi sensu lato* group associated with Lyme disease.

Specimen types, requirements and expected turn-around time

Test	Specimen required	Volume	Turn-around time	Comments
Lyme screen and blots	Serum	1 ml	Screen: 5 days Blot: 7 days	Submitted as in scope for ISO15189
CSF testing Immediate future	IN ALL CASES send Contemporaneous serum AND CSF ***with protein & IgG and IgM concentrations provided by referrer*** *or* CSF: no concentrations given	1 ml serum 0.5 ml CSF >0.5 ml CSF	 7 days 10-16 days‡	CE marked test Offered as out-of-scope under ISO 15189 pending additional data Times reflect the need for sending samples externally to provide protein/Ig levels.
PCR: Lyme disease	CSF Tissue	150 µl extra Punch biopsy/ 0.1 g tissue	7 days	
PCR for relapsing fever group Borrelia; including *B. miyamotoi.*	EDTA plasma	0.5 ml	7 days	Developmental only: call 01980 612348 to discuss first

‡RIPL is introducing dedicated equipment for undertaking these measurements locally. This will reduce turn-around times and the volume of CSF required. We will notify users when this is operational.

Lyme serology on serum samples

Screening ELISA test

The screening ELISA is unchanged, and is based on the commercial Immunetics C6 assay, which detects both IgM and IgG antibodies to the conserved IR6 region of the VlsE protein of *Borrelia burgdorferi sensu lato*. The VlsE protein is the main surface antigen expressed by the organism in the established infection, and has variable domains which the organism can rotate during the course of an infection to evade the host response. The IR6 domain is conserved throughout these changes, and is highly immunogenic so that antibodies to the synthetic epitope (the C6 peptide) are seen across all the variants. Samples that give a positive or equivocal result in the screening assay are confirmed by immunoblot. For

potential cases of Lyme disease where the sample has been taken within four weeks after onset, we recommend repeating the test 3-4 weeks later if the initial result was negative as seroconversion can be delayed.

Confirmatory immunoblot for Lyme disease

Samples with positive or equivocal screening ELISA results are confirmed using an immunoblot, with separate blots for IgG and IgM antibody responses to a selection of antigens found across the range of pathogenic strains in the *Borrelia burgdorferi sensu lato* group. Many of these antigens are plasmid mediated, and have multiple variants so the pattern of reactive analytes varies between patients because of variations in the causative organism and the patient's response. For this reason, the assay comprises antigens that widely shared across the group, as well as antigens identified in specific strains where a strong antibody response is mounted in some patients.

The ViraStripe system used from July 2012 to November 2017 uses printed blot strips for IgG and IgM, which are set up by robot and read on a separate densitometer. RIPL are changing to the modified ViraChip assay which uses the same antigens printed as spots in a 96 -well plate format. To improve both precision and to allow for particles obscuring any single spot, the antigens spots are triplicated for each antigen in the well. The assay is performed automatically using a dedicated high precision robot with the densitometer combined, and the results are interpreted by the built in software, avoiding any risk of subjective interpretation. Each plate includes control, alignment and cut-off spots. The system offers tighter control of inter-assay variability and a greatly increased throughput, which will assist with quality control, reducing repeat testing and turn-around times. In either system, a single reactive antigen (out of five) in the IgM assay is recorded as positive; two reactive antigens out of eleven are required for a definitive positive in the IgG blot. Blots are noted as positive when the density of the band or spots is equal to or greater than the density of the cut-off band included in the assay.

CSF testing

Contemporaneous paired serum and CSF samples are required for analysis of CSF antibodies. The European Federation of Neurological Sciences (EFNS) has produced detailed guidance on the diagnosis and management of neuroborreliosis. Laboratory confirmation of neuroborreliosis is based on demonstrating intrathecal synthesis of borrelia specific antibodies, as well as the cellular response. In practice this means that we need to compare the antibody profiles on serum and CSF samples taken at the same time, and to correct for the different concentrations of total IgM and IgG in serum and in CSF. This is done by calculating an antibody index from the concentrations of each in serum and CSF, together with the albumin concentrations and then comparing the responses to the different borrelia antigens between a serum diluted to bring the total Ig concentrations in line with those in the CSF. RIPL will perform these measurements if the values are not provided by the referring laboratory. *Any* antibody to a borrelia antigen detected in the CSF that is not present in serum, or which has a higher blot intensity than the corresponding antibody in the diluted serum is evidence of intrathecal synthesis of borrelia antibodies and supports the confirmation of neuroborreliosis. An undiluted serum blot analysis will automatically be performed to provide an assessment of the overall systemic serological response. The CSF and the neat serum results will be reported in full for all antigens represented on the blot system. The analyser software automatically determines whether a particular antibody result is positive or negative and no additional interpretation is permitted. If there is sufficient sample remaining, all CSF samples will also be tested by PCR.

CSF protein values

Unless the serum and CSF albumin, IgM and IgG concentrations are supplied by the requesting laboratory, RIPL will either arrange for testing by a local accredited laboratory (Southampton Immunology department), or in due course test internally as part of the service. The cost of these analyses will be deducted from the test charge when values are provided by the referring laboratory with the samples. In the immediate future testing will be undertaken for IgG and albumin concentrations only, and these values used to determine the serum dilution factor. RIPL is procuring a dedicated analyser to cover all immunoglobulins, albumin and total protein amongst other analytes, which will reduce the volume of CSF required considerably.

PCR testing for Lyme and other borrelia

PCR is of limited value in testing for Lyme disease as the organism is rarely present in the blood at the time the patient is seen. PCR can be useful for additional diagnostic support when used on CSF and on tissue biopsies for acrodermatitis chronica atrophicans or borrelial lymphocytoma. For other indications, please discuss with a RIPL consultant.

The present PCR for *Borrelia burgdorferi sensu lato* species will be replaced in the next few months with a new pan-borrelia assay based on a highly conserved region of the 16S RNA when validation data is complete. This is also suitable for identifying relapsing fever group Borrelia, which are present in the blood during the febrile episodes, including *Borrelia miyamotoi*. The assay is classified as developmental in this application, as only limited data is available in-house or worldwide on tick-borne relapsing fevers. An EDTA sample taken from a febrile patient should be submitted. Relapsing fever group borrelia also generate some antibody responses that are found in Lyme disease, and a C6 test and Lyme blot can be used for travellers from affected regions (Africa, Southern US and tropical America, SE Asia). Please discuss with a RIPL consultant on 01980 612348 if you wish to test for these organisms.

Clinical support

A clinical advice service for registered medical practitioners is available in working hours on 01980-612348. Technical queries and service questions including results raised by referring laboratories can be taken on the same number. Users may also contact us by email at lyme.ripl@phe.gov.uk.

RIPL does NOT offer any direct services for patients except through their medical practitioner, will not accept samples for Lyme testing unless submitted through a recognised pathology provider, and cannot provide results over the telephone to patients as the identity of the caller cannot be confirmed. RIPL staff will not interpret results from laboratories outside the NHS.

Yours sincerely

Dr Tim Brooks
Clinical Services Director &
Head of Rare & Imported Pathogens Laboratory
Public Health England