Public Health England

Protecting and improving the nation's health

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To all Clinicians requesting Lyme Testing

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Dear Doctor,

Re: Notes for clinicians on laboratory tests for Lyme disease

Lyme disease has many presentations, from the characteristic erythema migrans (EM) rash to facial palsy and other neurological symptoms. These notes are intended to help you select the right test to request and to interpret the results.

The Rare & Imported Pathogens Laboratory (RIPL) offers both serological testing for Lyme disease and for particular cases, PCR to detect the organism in a suitable sample. The laboratory does not provide a routine culture service because the organisms take several weeks to grow, and sensitivity is around 50% even on optimal samples such as skin biopsies. The new NICE guidance on Lyme disease (to be published in April 2018) gives guidance on testing. The available tests and specimens required are summarised in the table below.

Tests for acute Lyme disease

Testing is not normally required for patients with an acute EM rash, and is likely to be negative in the first 2-3 weeks after infection as the antibody response develops slowly. EM should be treated on clinical suspicion, and testing is not indicated unless there is doubt about the diagnosis. If a negative result is obtained, and Lyme disease remains a possibility, the test should be repeated after 4-6 weeks when an antibody response will have appeared.

Not all patients will develop a rash, and 25-30% present with other manifestations of Lyme disease. These patients should be tested, and if negative the test repeated to look for seroconversion on a later sample unless another cause for the condition has been identified in the meantime. Depending on the likelihood of Lyme disease, early treatment should be offered.

Specimen types, requirements and expected turn-around time

Test	Specimen required	Volume	Turn-around time	Comments
Lyme screen and blots	Serum	>0.5 ml	Screen: 5 days Blot: 7 days	Submitted as in scope for ISO15189
CSF testing by immunoblot Immediate future	IN ALL CASES send Contemporaneous serum AND CSF ***with protein & IgG and IgM concentrations provided by referrer**** *or* CSF: no concentrations given	>0.5 ml 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 Turn-around 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 e.g. *B. miyamotoi*	EDTA plasma	0.5 ml	5-7 days	Developmental only: call 01980 612348 to discuss first

*if the local laboratory cannot, or does not, perform the protein and Ig concentrations, RIPL will arrange for these to be performed by an external laboratory; †RIPL is introducing dedicated equipment for protein analysis which will halve this time; we will notify users when the enhanced service is available.

All samples are tested using a sensitive screening assay (the C6 IgM/IgG ELISA) and positives confirmed by IgM and IgG immunoblots.

Early disseminated Lyme disease

These patients should be tested, and if negative the test repeated to look for seroconversion on a later sample unless another cause for the condition has been identified in the meantime. Depending on the likelihood of Lyme disease, early treatment should be offered.

All samples are tested using a sensitive screening assay (the C6 IgM/IgG ELISA) and positives confirmed by IgM and IgG immunoblots.

Late disseminated Lyme disease

Patients with possible or likely late disseminated or established Lyme disease should be tested serologically. Repeat samples are not necessary if the symptoms have been present for two months or more. The specimens are tested by ELISA and immunoblot as above.

PCR testing

PCR is of limited value in routine testing for Lyme disease as the organism is only present in the blood in early stages of the disease, and is largely found in the affected tissues. In early disseminated disease, PCR may be positive in the blood, but a negative test is of no value in excluding Lyme disease. Punch biopsies of an EM rash have a sensitivity of around 50%, limited by the chance of a single biopsy hitting exactly the site with a significant number of organisms.

PCR may be of value in disseminated Lyme disease for confirmation of acrodermatitis chronica atrophicans in a biopsy of the lesion, and in neuroborreliosis and arthritis. Biopsies should be submitted as *fresh tissue* in a sterile container, ideally with a drop of sterile saline to prevent the tissue drying out. In neuroborreliosis involving the CNS up to 10% of cases may be PCR positive on a CSF sample; a negative *does not* exclude the diagnosis. Synovial fluid may occasionally be positive by PCR; again a negative result does not exclude the diagnosis.

PCR may be useful in the diagnosis of other Borrelia infections caused by relapsing group organisms: please contact the laboratory for advice.

Testing CSF samples for Lyme disease

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(1). 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.

Please note that it is extremely difficult or impossible for us to obtain meaningful results for CSF antibody measurements without a contemporaneous serum sample (taken on the same day, or *in extremis* within a day either side), and largely pointless for you and the patient. There are a few general items to note about CSF testing in Lyme disease:

1. A negative serum antibody test does NOT exclude a positive CSF in EARLY neuroborreliosis, especially in children.
2. Localised neuroborreliosis may not give rise to detectable antibodies in CSF so a negative CSF blot does not exclude this as a cause of e.g. facial palsy in a child.
3. As small proportion of late neuroborreliosis cases may have negative blood serology, but positive CSF antibodies. Please call to discuss testing if you think this may apply to your patient.
4. Antibodies to OspC in CSF are associated with EBV in a proportion of cases, especially in children so if this is the only evidence of Lyme disease please consider the differential diagnosis.
5. P39 antibodies cross-react with syphilis so consider this possibility in interpreting result if this antibody result is not supported by other borrelia antibodies or clinical evidence.
6. P21 antibodies are strongly associated with neuroborreliosis, both in CSF and serum
7. P17 IgG antibodies persist for a long time, and are also found in CSF frequently in neuroborreliosis.
8. The pattern of intrathecal antibodies is very variable, and often does not mirror the serum response: these differences favour the diagnosis of neuroborreliosis.

Further advice

Clinicians are welcome to speak to one of the RIPL clinicians in working hours on 01980 612348 to discuss any aspect of testing for Lyme disease or the results of tests already performed. Clinicians may also contact us 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.

1. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol. 2010;17(1):8-16, e1-4.

Yours sincerely

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