INESSS
LE SAVOIR PREND FORME

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TWO-TIERED SEROLOGY

Complementary laboratory test for the diagnosis of Lyme disease

The information below is provided as a guide only and should not replace the clinical judgment of the clinician who performs the activities reserved under an act or a regulation. The findings result from a systematic process and are supported by the scientific literature and the knowledge and experience of Québec clinicians, experts and patients. For further details, go to **inesss.qc.ca**.

PRACTICAL INFORMATION

1st tier	ELISA
2end tier	Western blot
Sequence	Only specimens that are positive or equivocal on ELISA are subjected to Western blot
Turnaound time	• 5 business days for ELISA screening • 10 business days if Western blot proves necessary
Type of specimen	Blood (serum)

TARGET POPULATION AND OBJECTIVES

- Patients in whom Lyme disease is suspected after the history is taken, including an assessment of the level of risk of tick exposure, a complete physical examination and consideration of other clinical conditions (refer to the Lyme disease diagnostic support tool for further information).
- Serological tests are aimed at detecting the presence of antibodies in the bloodstream directed against proteins of bacteria belonging to the *Borrelia burgdorferi* complex.

The diagnosis is based on the entire clinical picture, which serological tests serve to complement.

ADVANTAGE

- Dectection of antibodies against specific bacterial proteins.

LIMITATIONS

- Indirect detection method (depends on the development of an immune response).
- The results are not markers of therapeutic activity or effectiveness.
- The interpretation of the results is subjective.
- Possibility of false positives for IgM (Western blot) if the specimen is obtained more than 4 to 6 weeks after the onset of symptoms.
- The test cannot distinguish between an active bacterial infection and a past infection.

INDICATION

Refer to the Lyme disease diagnostic support tool for further information.

Indicated	Not indicated
Manifestations of the early or late disseminated stage when: • the clinical picture is consistent with Lyme disease and • there is concurrently no typical isolated EM	If isolated EM, since the sensitivity is too low at this stage and the result would probably be negative. • Exception: patients with isolated EM that is not typical and with signs suggestive of the disseminated stage For assessing the effectiveness of antibiotic therapy because antibodies can remain in the bloodstream for a long time.

ELISA: enzyme-linked immunosorbent assay; **EM:** erythema migrans; **IgG:** immunoglobulin G; **IgM:** immunoglobulin M

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DIAGNOSTIC VALUE OF TWO-TIERED SEROLOGY

- There are different two-tiered serological testing approaches, depending on the type of test performed, the most common approach forming the subject of this document, namely, ELISA followed by Western blot.
- In general, the sensitivity of the two-tiered serological tests used in North America:
  - → Is low at the onset of the infection (25% to 60%, depending on the study and the approach);
  - → Increases gradually with time (40% to 100%, depending on the manifestation, the study and the approach);
  - → Is high in patients with Lyme arthritis (95% to 100%, depending on the study and the approach);
  - → Is low for Lyme disease contracted in Europe, Asia or North Africa because the *B. burgdorferi* genospecies are different. If need be, different Western blot tests are used (the differences concern the choice of antigens and positivity criteria).

There remains some uncertainty regarding the diagnostic value of two-tiered serology because the designs of the available scientific studies are poorly suited to demonstrate it.

- The following table shows the diagnostic value of a two-tiered serological testing approach similar to the one used in Québec[1].

Manifestations	Sensitivity of the tests used in North America	
	LD contracted in North America	LD contracted in Europe
LOCALIZED STAGE		
Erythema migrans	27% to 40% (4 studies, N = 606) Level of scientific evidence: low[2]	10% and 20% (2 studies, N = 40) Level of scientific evidence: insufficient[2]
EARLY DISSEMINATED STAGE		
All manifestations combined	No study found	No study found
Systemic symptoms	29% and 43% (1 study, N = 60) Level of scientific evidence: insufficient[2]	No study found
Neuroborreliosis	47% to 90% (3 studies, N = 59) Level of scientific evidence: insufficient[2]	20% and 40% (2 studies, N = 25) Level of scientific evidence: insufficient[2]
Lyme carditis	No study selected	No study found
LATE DISSEMINATED STAGE		
All manifestations combined	No study found	50% (1 study, N = 10) Level of scientific evidence: insufficient[2]
Lyme arthritis	95% to 100% (3 studies, N = 184) Level of scientific evidence: low[2]	67% (1 study, N = 15) Level of scientific evidence: insufficient[2]

Type of controls	Specificity[3] of the tests used in North America	
	LD contracted in North Amerrica	LD contracted in Europe
Healthy	99% to 100% (5 studies, N = 3454) Level of scientific evidence: low[2]	100% (1 study, N = 100) Level of scientific evidence: insufficient[2]
Healthy or with some other condition	99% to 100% (5 studies, N = 4034) Level of scientific evidence: low[2]	No study found

1. The diagnostic values are shown for the combination of an ELISA in which the antigen is a synthetic VlsE protein peptide or the entire protein and Western blot, regardless of the kit used. For additional information about the kits used by the LSPQ, consult the guide de services.
2. For additional information, consult the state-of-knowledge report on the diagnostic value of laboratory tests (État des connaissances – Valeur diagnostique des analyses de laboratoire).
3. In the available studies that were selected, the specificity of two-tiered serology was studied only in cohorts of healthy individuals or individuals with clinical conditions unrelated to Lyme disease. The specificity reported is that obtained in the largest number of controls.

LSPQ: Laboratoire de santé publique du Québe; **ML:** Lyme disease; **N:** number of participants.

INTERPRETATION OF RESULTS

The results of two-tiered serology should be interpreted in light of the clinical picture.

ELISA	
General considerations	• IgG and IgM are tested for together or separately, depending on the kit. • In general, the kits can be used for all of the complex's bacterial genospecies.
Reference values	• Vary according to the kit used.
Interpretation of results	• **Positive or indeterminate result** in a patient **with manifestations suggestive** of Lyme disease: → Risk of a false-positive result. The result should be confirmed with Western blot. • **Negative result** in a patient **with manifestations suggestive** of Lyme disease: → **Does not rule** out Lyme disease without an assessment of the entire clinical picture, since the tests depend on the development of an immune response.

Western Blot	
General considerations	• Is performed only on specimens that are positive or equivocal on ELISA. • IgG and IgM are tested for separately. IgG results are more reliable, so IgG testing is done first. • IgM testing is not done if the IgG result is positive or if the symptoms had been present for more than 6 weeks at the time of specimen collection. **The kits differ according to the complex's bacterial genospecies, hence the need, when ordering, to indicate the continent or continents where the disease may have been contracted.** • The antigens detected appear to vary according to the geographical location and when the infection was contracted, among other things. There are no scientific data currently available for assessing the clinical utility of the profile of the detected antigens[1]. **The profile of the detected antigens should not be used to guide the clinical management of patients with suspected Lyme disease.**
Reference values	• Positive IgG test: ≥ 5 bacterial antigens in 10 are recognized. • Positive IgM test: ≥ 2 bacterial antigens in 3 are recognized.
Interpretation of results	• **Positive IgG result, regardless of the IgM result:** → Is an element in favour of the diagnosis and should be used to complement the clinical picture. • **Positive IgM result and negative IgG result:** → Strong possibility that this is a false-positive result, especially if the patient has had symptoms for more than 6 weeks; → If Lyme disease is strongly suspected, consider talking to an experienced colleague or a specialist to determine if serology testing needs to be done again 4 to 6 weeks later. • **IgM and IgG results both negative:** → **This does not rule out** Lyme disease without an assessment of the entire clinical picture because: - The tests depend on the development of an immune response; - Not all of the various *B. burgdorferi* genospecies and their different strains induce an immune response against the same antigens (e.g., European strains).

1. For further information, consult the report concerning the clinical utility of the Western blot antigen profile. (Avis – Utilité clinique du profil d'antigènes détectés par immunobuvardage).

ELISA: enzyme-linked immunosorbent assay; **IgG:** immunoglobulin G; **IgM:** immunoglobulin M.

OTHER INFORMATION ON TWO-TIERED SEROLOGY

Two-tiered serology combining two ELISA tests	• The available data suggest that this test combination: → Has a diagnostic value similar to that of ELISA combined with Western blot; → Could be advantageous at the onset of the infection. ! *However, the data were obtained from a small number of participants, and the levels of evidence were considered insufficient.* • Is an acceptable alternative to serology combining ELISA and Western blot, according to the Centers for Disease Control and Prevention (Mead *et. al.*, 2019). • The Lyme Disease Diagnostic Working Group agreed with the CDC's recommendation on the condition that the efficacy of this approach is fully validated in the Canadian context (Hatchette and Lindsay 2020).
Laboratories that perform two-tiered serology	• Public laboratories and a number of private laboratories perform these tests and interpret the results in accordance with the CDC's recommendations. • Some private laboratories: → Do not perform the two tiers of the serology in sequence; – Risk of false positives if Western blot is used alone → Use Western blot tests that differ from those recommended in at least one of the following respects: – The choice of antigens – The number of antigens used – The positivity criteria used (possibility of a large number of false positives[1])
The so-called chronic form of Lyme disease	• We cannot, from the available scientific data, rule on the plausibility that the bacteria responsible for Lyme disease can, directly or indirectly, cause persistent, general systemic symptoms. • Although the condition of patients with such symptoms is attributed to Lyme disease by some, the current state of knowledge shows that: → The bacteria that cause Lyme disease are not the only possible etiological agents in such cases; → Other, non-infectious mechanisms could also be involved, as could other clinical conditions. • Some stakeholders suggest interpreting Western blot results for Lyme disease in a different manner than that recommended by the CDC, but there is no evidence to support this approach.

RECOMMANDATIONS

- All of the recommendations regarding two-tiered serology are shown in the diagnostic support tool for localized and disseminated Lyme disease. The rationale for these recommendations is presented in the report in support of this tool (Lyme Disease, from diagnosis to treatment, at the localized and disseminated stages).

MAIN REFERENCES

Institut national d'excellence en santé et en services sociaux (INESSS). Du diagnostic au traitement de la maladie de Lyme aux stades localisé et disséminés. Rapport en soutien aux outils d'aide à la décision clinique sur le diagnostic et le traitement. Québec, Qc: INESSS; 2019. Available at: https://www.inesss.qc.ca/en/publications/publications/publication/maladie-de-lyme.html.

Institut national d'excellence en santé et en services sociaux (INESSS). Valeur diagnostique des analyses de laboratoire pour la maladie de Lyme aux stades localisé et disséminés. Québec, Qc: INESSS; 2021. Available at: https://www.inesss.qc.ca/en/publications/publications/publication/maladie-de-lyme.html.

Institut national d'excellence en santé et en services sociaux (INESSS). Utilité clinique des différents profils d'antigènes détectés par immunobuvardage dans le contexte de la maladie de Lyme aux stades disséminés. Québec, Qc: INESSS; 2021. Available at: https://www.inesss.qc.ca/en/publications/publications/publication/maladie-de-lyme.html.

Institut national d'excellence en santé et en services sociaux (INESSS). Maladie de Lyme dite chronique : État actuel des connaissances scientifiques et portrait des différentes perspectives. Québec, Qc: INESSS; 2021. Available at: https://www.inesss.qc.ca/en/publications/publications/publication/maladie-de-lyme.html.

Institut national d'excellence en santé et en services sociaux (INESSS). Maladie de Lyme et symptômes persistants : regard sur les connaissances actuelles et recommandations pour l'amélioration de la prise en charge. Québec, Qc : INESSS ; 2021. Available at: https://www.inesss.qc.ca/en/publications/publications/publication/maladie-de-lyme.html.

Mead P, Petersen J, Hinckley A. Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep 2019;68:703. DOI: http://dx.doi.org/10.15585/mmwr.mm6832a4.

Hatchette T et Lindsay R. Modified two-tiered testing algorithm for Lyme disease serology: the Canadian context. Can Commun Dis Rep 2020;46(5):125-31.

CDC : Centers for Disease control and Prevention ; **ELISA :** enzyme-linked immunosorbent assay

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