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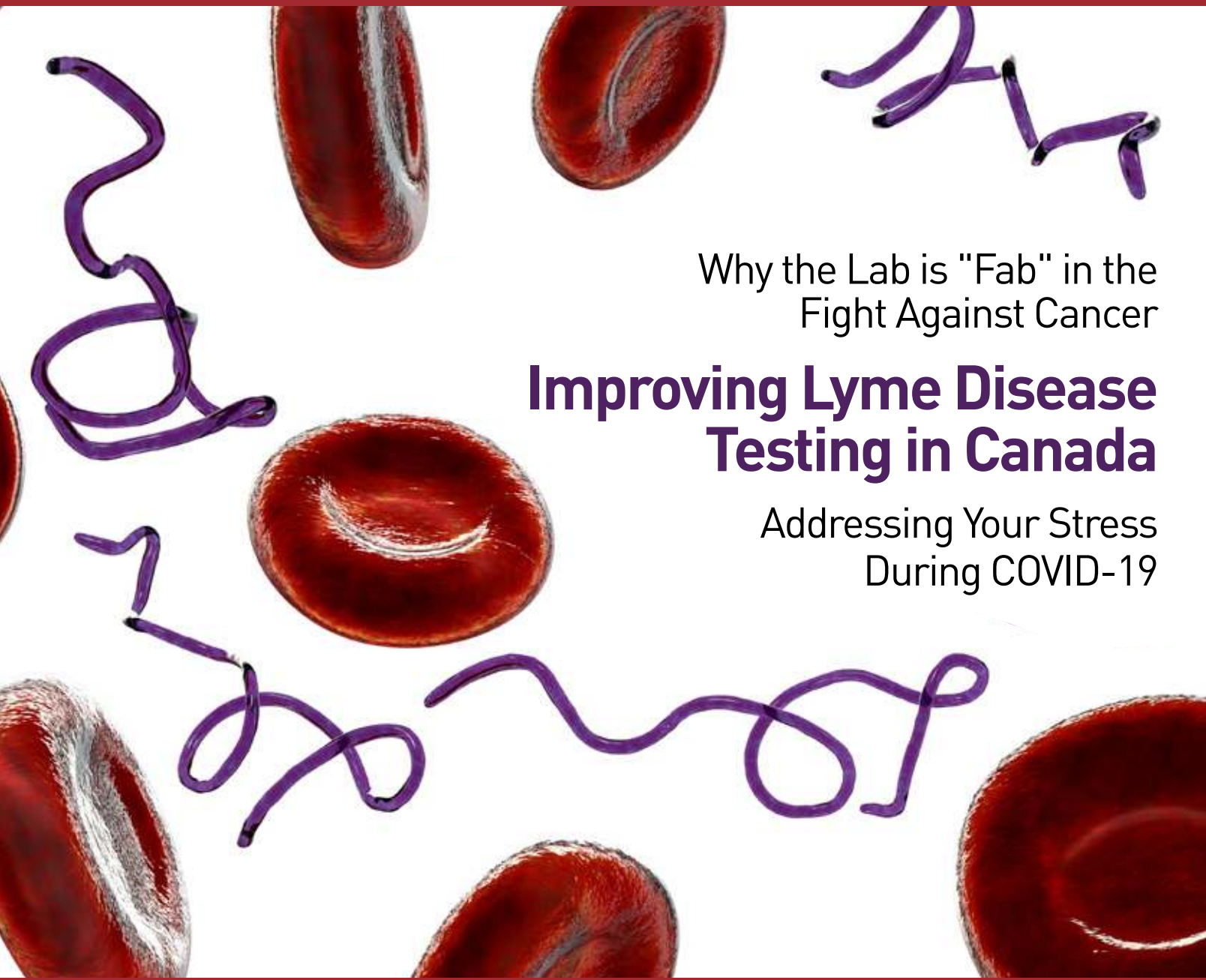

Why the Lab is "Fab" in the Fight Against Cancer

Improving Lyme Disease Testing in Canada

Addressing Your Stress During COVID-19

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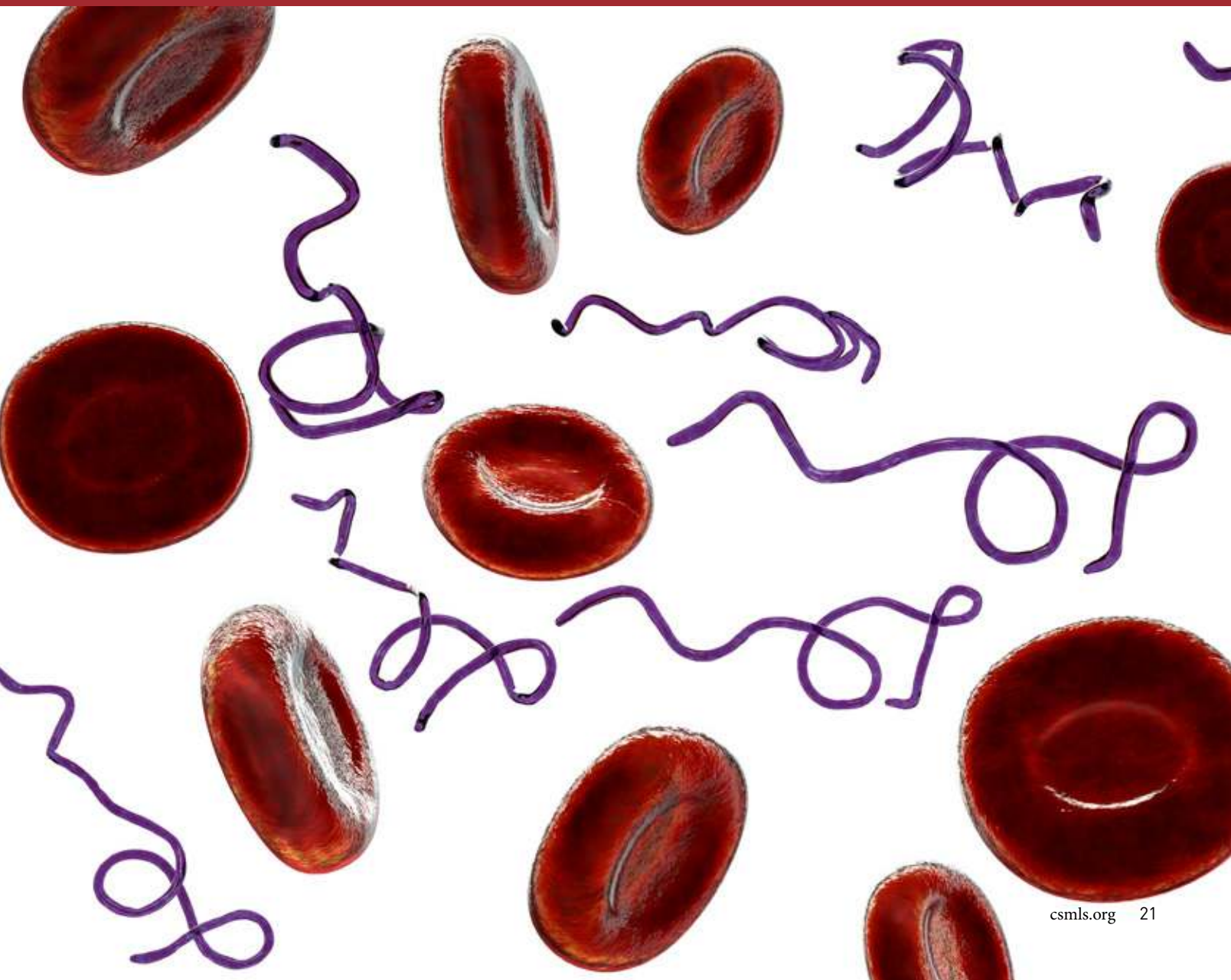
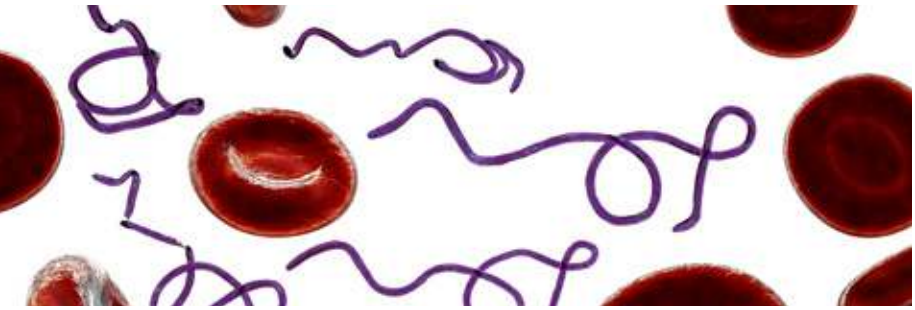
IMPROVING LYME DISEASE TESTING IN CANADA

The current diagnostic testing approach for Lyme disease is least reliable for early infections, but that is when treatment with antibiotics is most effective. Canadian researchers are validating a modified testing approach that may help improve patient outcomes. »





The current approach for laboratory diagnosis of Lyme disease is a two-tiered serological test that aims to detect antibodies to *B. burgdorferi.* It consists of an enzyme immunoassay (EIA), followed by a confirmatory immunoblot.

The Disease

Lyme disease is caused by an infection with *Borrelia burgdorferi* spirochetes, which are transmitted to humans by blacklegged ticks.[1] The primary vectors for Lyme disease in Canada are *Ixodes scapularis* in eastern and central Canada and *Ixodes pacificus* in British Columbia. Infected tick populations in Canada are endemic across Nova Scotia, and in parts of New Brunswick, Ontario, Quebec, Manitoba and British Columbia.[2]

Lyme Disease Prevalence

In Canada, Lyme disease cases have risen from 144 in 2009 when it was first reported nationally to 2,025 in 2017. The real numbers are likely higher.[1,11] As the geographic spread of infected ticks continues to expand, more Canadians will be at risk of contracting it.[12]

In the United States, 300,000 new cases are diagnosed annually.[13]

When a tick bites a human for a blood meal and stays attached for more than 36 hours, the corkscrew-shaped spirochetes migrate from the tick's hindgut to its salivary glands and into human skin. From there, they travel into the bloodstream and make their way into joints and tissues. Early signs and symptoms of Lyme disease may include an erythema migrans rash and flu-like symptoms such as fever, chills, headache, fatigue, joint and muscle aches, and swollen lymph nodes. These symptoms can become more severe if they are left untreated. Additional symptoms, including facial paralysis, heart disorders, neurological disorders and arthritis with severe joint pain and swelling also may occur in later disease stages.[3]

The Test

The current approach for laboratory diagnosis of Lyme disease is a two-tiered serological test that aims to detect antibodies to *B. burgdorferi*. It consists of an enzyme immunoassay (EIA), followed by a confirmatory immunoblot. Most provincial public health and hospital labs perform EIAs. Several formats are available: Some include whole cell sonicates (WCS) of the laboratory strain of *B. burgdorferi* B31, and more recent EIAs contain synthetic peptides of common regions found in multiple *B. burgdorferi* strains. Examples include the surface lipoprotein variable major protein-like sequence expressed (VlsE), the invariable region 6 of VlsE (C6), and the conserved amino-terminal portion of outer surface protein C (C10). The specificity of these newer EIAs is better than WCS, but still not sufficient for their use as stand-alone tests.[4] Provincial public health labs and the National Microbiology Laboratory (NML) in Winnipeg, Manitoba, perform immunoblot testing. Immunoblots take longer to complete, turnaround times are typically longer than with EIAs[5,6] and scoring the blots can be subjective.[6]

The usefulness of a lab test is measured by sensitivity and specificity (see Box 1). "The sensitivity and specificity of the

> Despite Canada having an overall low prevalence of Lyme disease, about three cases per 100,000 people, it's important to continue to test patients with a clinical suspicion of the disease in endemic areas.
>
> *– Prameet Sheth, PhD.*

Measuring Laboratory Test Performance[14]

Sensitivity: Measures how often a test correctly generates a true positive result for patients who *have* the condition. A test with high sensitivity will identify almost all patients who have the disease and not find many false negatives. For example, if you test 100 positive samples with a test that has a sensitivity of 90%, it will correctly identify 90 that are truly positive and 10 that are false negatives.

Specificity: Measures how often a test correctly generates a true negative result for patients who *do not have* the condition. A test with high specificity will correctly rule out most patients who do not have the disease and not find many false positives. For example, if you test 100 negative samples with a test that has a specificity of 90%, it will correctly identify 90 that are true negatives and 10 that are false positives.

Box 1

current testing approach for Lyme disease depend on the prevalence of the disease in a population, as is true for serological tests for other infectious diseases," says Prameet Sheth, PhD, a clinical microbiologist at Kingston Health Sciences Centre and assistant professor in the Department of Pathology and Molecular Medicine at Queen's University in Kingston, Ontario. "Despite Canada having an overall low prevalence of Lyme disease, about three cases per 100,000 people, it's important to continue to test patients with a clinical suspicion of the disease in endemic areas.

"The current test for Lyme disease is very good at detecting late acute Lyme disease, but not very good for early acute cases," says Sheth. Case in point: A recent systematic review found that the sensitivity of the standard method approaches 100 per cent in detecting late stages of infection but is less than 50 per cent in identifying early localized infections.[7]

How well the current testing approach can accurately detect antibodies to *B. burgdorferi* in circulating blood depends on several factors: the quantity of bacteria transmitted by an infected tick, the immune response mounted by the patient, how long the patient has been infected and when the sample was obtained relative to disease stage.

The Future

A new approach called the modified two-tiered testing algorithm has shown potential for improving sensitivity without compromising specificity. It uses a second EIA instead of an immunoblot. Developed in the United States, where Lyme disease has a much higher prevalence than in Canada, it has been approved by the U.S. Food and Drug Administration[8] and endorsed by the Centers for Disease Control and Prevention[9] as an acceptable alternative to the standard approach. The modified two-tier approach uses combinations of EIAs, such as WCS followed by C6, VlsE followed

Advantages and Disadvantages of the Modified Two-Tier Testing Algorithm Compared to the Standard Algorithm for Lyme Disease*

ADVANTAGES	DISADVANTAGES
• Improved sensitivity; detects 25% more early cases • Less labour-intensive • Less subjective • Faster turnaround time (TAT) as both EIA tests are performed locally • Faster TAT facilitates acute and convalescent testing in patients with non-erythema migrans early disease	• Patients with erythema migrans will still require antibiotic treatment since the test sensitivity is still less than 90% • Specificity in areas of low prevalence unclear • Potential for reduced specificity with some polyvalent EIAs means the standard method may still be beneficial in patients with Lyme arthritis

*Adapted from Hatchette T, Lindsay LR. *Can Comm Dis Rep.* 2020;46(5):125–31.[4]

by C6, C6 followed by VlsE, or VlsE/C10 followed by WCS.

A group of Canadian Lyme disease experts, known as the Lyme Disease Diagnostics Working Group of the Canadian Public Health Laboratory Network, recently reviewed the scientific literature. They examined the performance of different EIAs used in the standard and modified two-tier testing protocols in areas where infected blacklegged ticks are highly endemic. The investigators found that the modified approach was consistently more sensitive in detecting *B. burgdorferi* infections across all Lyme disease stages – especially early localized disease – compared to the current testing approach. They observed improvements in sensitivity without significant loss of specificity, regardless of the combinations of EIAs used.[4]

"We were quite excited to see that the modified approach detected more early cases of Lyme disease because earlier detection means better patient outcomes," says L. Robbin Lindsay, PhD, senior study author and research scientist with the Public Health Agency of Canada, Zoonotic Diseases and Special Pathogens section of the NML. "It's less laborious to do two EIAs, which can be done locally in a day or two, rather than an EIA followed by a complex immunoblot. Shorter turnaround times may help doctors solve more challenging cases." The modified approach is also less expensive for the health care system, but obtaining the most accurate results for patients is the priority, he says.

So far, Lindsay and colleagues have validated the modified two-tier algorithm using 447 samples from patients in Nova Scotia, the province with the highest prevalence of Lyme disease in Canada. They found that the modified approach detected 25 per cent more cases of early localized infections than the standard method, with a high specificity of 99.5 per cent.[10]

Next, Lindsay and colleagues plan to validate the modified two-tier algorithm using patient samples banked during the 2020 tick season from different regions of Canada. They will run the second EIAs and review patients' charts to compare its performance to the standard method. "Validation with data from other regions is important before making a recommendation to change testing guidelines," says Lindsay. "We may find lower specificity and more false positives in areas of low endemicity."

> "Validating the two-EIA approach will take time, but if the data shows that it performs as well as or better than the current test, we will recommend rolling it out within the next year or two. The goal is to help doctors treat only the patients who need to be treated, as quickly as possible."
>
> *– L. Robbin Lindsay, PhD.*

Even if the new approach is adopted in the future, immunoblot testing will still play a role in patients who may have become infected while travelling in Europe or Asia, or have arthritis from suspected late-stage Lyme disease. Immunoblots will also help solve suspected false positive cases where serology results do not line up with symptoms.[4]

"Validating the two-EIA approach will take time, but if the data shows that it performs as well as or better than the current test, we will recommend rolling it out within the next year or two," Lindsay says. "The goal is to help doctors treat only the patients who need to be treated, as quickly as possible."

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