



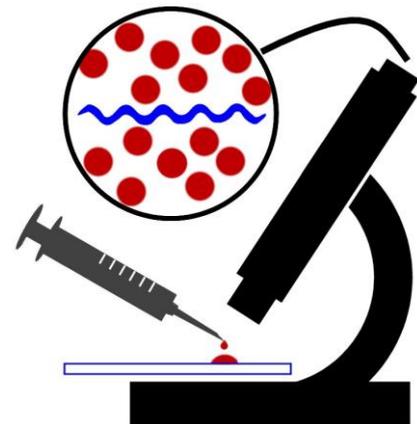
Immunofluorescence microscopy for Lyme borreliosis

At present, UK NHS testing fails to detect Lyme in an unknown number of patients which could amount to tens of thousands each year. These False Negative tests have potentially serious consequences for patients left infected but untreated.

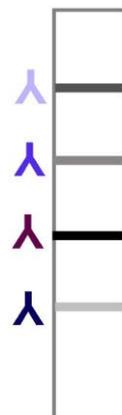
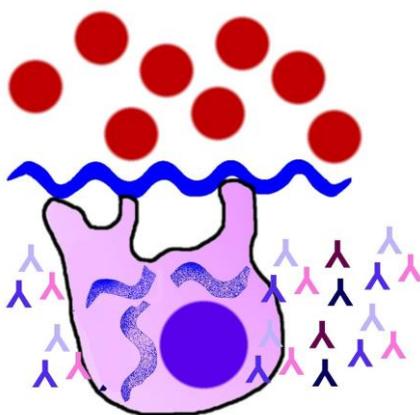
The main test currently used by the NHS does not attempt to detect the bacteria which causes Lyme, but instead looks for antibodies to the bacteria. This may be rational for testing relatively recently infected patients, but for people that have long-standing, chronic infection, it is a poor choice. *Borrelia burgdorferi* have multiple strategies to confuse and evade the immune system which can result in antibody levels dropping below the threshold of indirect, antibody tests.

Testing for Lyme borreliosis

The Gold Standard for diagnosing infectious diseases has always been direct detection of the infecting organism. This often requires a combination of processes which include sampling, culturing, then detecting the infecting organism by methods such as microscopy, antibody staining and PCR. If the organism is detected, these methods tell the pathologist that the infection is present and viable.



A different method is used by the NHS. Instead of trying to isolate the borrelia spirochaetes which cause Lyme borreliosis, they use indirect tests to look for antibodies produced by the patient's immune system. It is a useful method if the infection is at a certain stage when the number of infecting bacteria are could still be relatively low but antibodies are high enough to detect.



Indirect testing

Cells of the immune-system break-down invading bacteria and produce antibodies matching its component proteins. The presence of specific antibodies can be shown as bars on special test strips. These indicate that the infection is, or was present, and that the immune system responded.

Lyme Borreliosis and Fluorescent Antibody Staining

Albert Hewett Coons was an American physician, pathologist, and immunologist. He was the first person to conceptualize and develop immunofluorescent techniques for labelling antibodies in the early 1940s. (https://en.wikipedia.org/wiki/Albert_Coons)



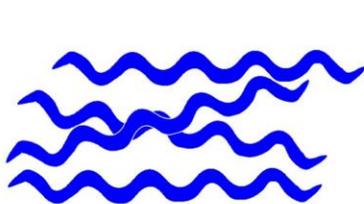
To the present day, many thousands of antibodies have been produced and are commercially available to research for identifying specific bacteria and other cells.

This long established and proven method has applications in the diagnosis and study of Lyme borreliosis. Commercial suppliers offer dozens of anti-*borrelia burgdorferi* antibodies. Some detect a single protein found in the spirochaete (monoclonal) others detect multiple proteins (polyclonal).

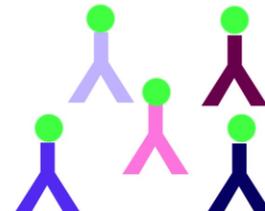
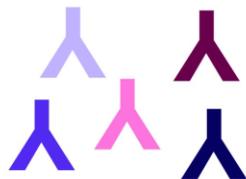
Direct detection of borrelia burgdorferi by Immunofluorescence

Polyclonal fluorescent antibodies for detection of borrelia proteins are made by:

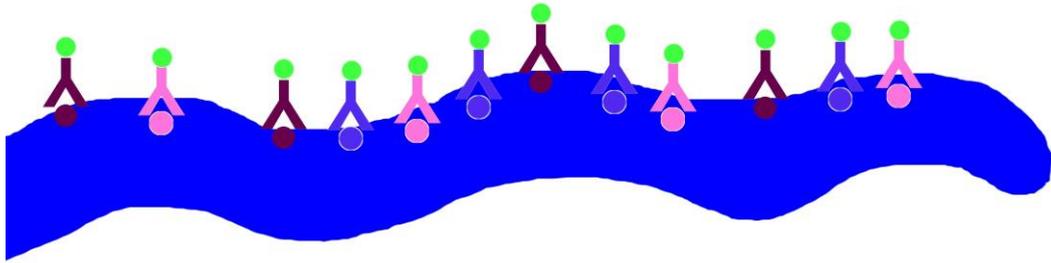
growing borrelia spirochaetes in a laboratory	which are sonicated or pulverised	then injected into	a host animal
---	-----------------------------------	--------------------	---------------



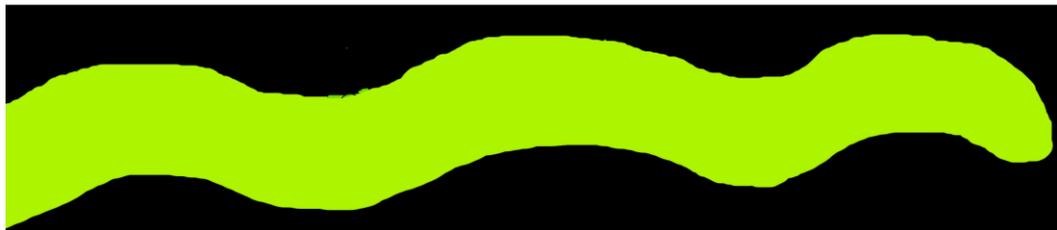
The animal's immune system produces antibodies to the various proteins of the borrelia.	The antibodies are collected and a fluorescent protein is attached to them (conjugated)
---	---



When the fluorescent antibodies are added to a sample containing the target organism, the antibodies attach to their matching proteins. These 'label' the organism with fluorescent proteins:



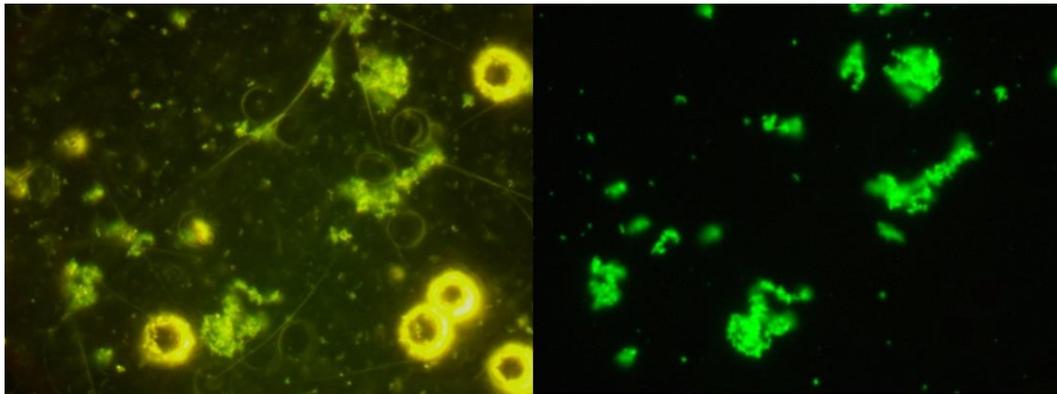
A microscope equipped with the correct filtering can observe just the fluorescence of a sample. When *fluorescein isothiocyanate* (FITC) is used as the fluorescent conjugate, the correct filtering means shining only blue light on the sample, then observing only the green light emitted as fluorescence:



There are some limitations to the method. For example, if a different spirochaete such as *treponema pallidum* which causes syphilis, were present in a sample from a suspected Lyme patient, some antibodies might attach to those organisms because they have some very similar proteins. This is known as cross-reactivity.

Borrelia burgdorferi and other Lyme borreliosis species have multiple strategies to evade detection by the immune system. Therefore the borrelia bacteria in a sample may be hidden from antibodies, whether those antibodies are from the patient's own immune system or are added as part of a laboratory test. This phenomenon can prevent fluorescent antibody staining from working. However, when a patient's blood sample is cultured for a period, the borrelia changes its morphology. The organism grows and sometimes it forms colonies. Then the bacteria exposes proteins which antibodies can attach to, making fluorescent antibody staining effective.

Below is a micrograph which shows a normal darkfield microscopy image of a blood sample which was cultured for a few days in BSK medium for *borrelia*. Red blood cells can be seen as bright yellow circles as well as long strands of fibrin and some other agents. On the right is a micrograph of exactly the same field of view with FITC fluorescence filtering, demonstrating the presence of borrelia spirochaete proteins as bright green agents. The red blood cells, fibrin and other agents are no longer visible.



A total of 8 donor samples were processed by the above method and all had similar results. All 8 donors have had Lyme borreliosis for years or even decades, with numerous symptoms and history correlating to the disease. All had positive Lyme tests from laboratories abroad. All had negative NHS tests for Lyme and their infection was not treated by the NHS.

Other techniques include binding unconjugated antibodies to target proteins then attaching a fluorescent probe to the antibody afterwards. These methods can be used on any tissues or fluids where the target organism is found.

Below are more examples of direct detection of borrelia burgdorferi with fluorescent antibody staining. More microscopy can be seen here:

<http://counsellingme.com/microscopy/FITCJuly2016.html>

<https://www.youtube.com/watch?v=COHyy6i5deQ>

And more VIRAS publications can be seen here:

<http://counsellingme.com/VIRAS/VIRAS.html>

