Abstracts Najaarsvergadering 2023

A diagnostic algorithm for Lyme neuroborreliosis diagnostics

Winnaar'Beste abstract Najaarscongres'

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The diagnosis of Lyme neuroborreliosis (LNB) can be complicated. The diagnosis is made based on clinical symptoms and must be supported by laboratory diagnostics. Laboratory diagnostics of LNB primarily focuses on the detection of intrathecally produced *Borrelia*-specific antibodies, although other routine and specific cerebrospinal fluid (CSF) and serum parameters can also be helpful. None of the available parameters, however, can independently confirm active LNB, and a validated diagnostic algorithm for LNB diagnostics is currently lacking.
In this retrospective cross-sectional study, seven commercial antibody tests were evaluated, followed by a multiparameter analysis to explore the added value of other CSF and serum parameters for LNB diagnostics. In total, 156 patients were included and classified as 'definite' (n=10), 'possible' (n=7), or non-LNB patient (n = 139) according to European neurology guidelines.
The sensitivity of the antibody tests varied between 47.1% and 100% and the specificity varied between 95.7% and 100%. Combining antibody test results with those of other routine and specific CSF and serum parameters increased the sensitivity (range: 94.1% to 100%), although at the expense of the specificity (range: 92.8% to 96.4%), which was slightly lower. The key parameters, in addition to intrathecal *Borrelia*-specific antibody detection, include two-step serology on serum, CXCL13 measurement in CSF, blood-brain barrier functionality combined with total antibody synthesis in CSF, and CSF-cell count.
This study showed that a multiparameter strategy adds value to LNB diagnostics. A standardized diagnostic algorithm for improved LNB diagnosis based on our finding should ideally be validated in a collaborative prospective study.

Fungi emerge from the shadow of bacteria WHO-fungal priority list / Mycology surveillance platforms setup –RIVM

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Fungi are an increasing public health problem. Due to changes in their habitat (the environment) and new human medical treatments, such as antibiotics and drugs that affect the immune system, there is an increase in patients with serious invasive fungal infections. Secondary fungal infections are also reported in patients admitted to intensive care with

influenza or coronavirus infections (SARS-CoV2), with even previously healthy patients developing invasive aspergillosis. Not only an increase in the number of infections with Aspergillus, but also the rapidly developing resistance to available anti-fungal medications is a growing problem.

In 2022, WHO has published a fungal priority list to facilitate the research of mycology. As the national institution of public health and environment, we aim to setup a mycology molecular surveillance platform to gain a better insight into the occurrence of (resistant) yeasts and molds in humans and the environment and to map the transmission routes from their habitat to the patient. This set-up fits in with a more general pursuit of "preparedness and response" for the emergence of new (resistant) pathogenic microorganisms with the aim of developing effective interventions quickly. On the current state, we are taking *A. fumigatus* as an example for azole resistance research and is establishing advanced technics and sequencing facilities.

Multiplex qPCR assays for the detection of *Ixodes ricinus*-borne pathogens

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Tick-borne pathogens (TBPs), other than *Borrelia burgdorferi* s.l., are emerging, although their potential to cause disease in humans remains unclear. Adequate direct detection methods such as qPCR are lacking in routine clinical or research settings. Here, three multiplex qPCRs are used for detection of TBP DNA in ticks and host species material. The aim is to evaluate these qPCRs for the detection of TBPs in human samples.

We evaluated the analytical specificity, sensitivity, and robustness of multiplex qPCRs for the detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *B. miyamotoi*, *Babesia* species, Spotted Fever Group *Rickettsiae*, candidatus *Neoehrlichia mikurensis*, *Spiroplasma ixodetis*, and *Bartonella* species in human samples. Due to the scarcity of positive human samples, negative human matrices were spiked with synthetic controls.

The three qPCRs performed within the limits of dealing with microorganisms for which confirmed patient materials are scarce or non-existent. Spiking negative clinical samples with these micro-organisms showed that the detection of the TBPs is not inhibited by matrices of human origin such as blood or cerebrospinal fluid. There was acceptable sensitivity, good inter-assay variability and absence of cross-reactivity when multiplexing the different pathogens that makes them potentially suitable for human diagnostics.

The qPCRs evaluated in this study are suitable for laboratory diagnostic assessment of human clinical samples. Further clinical validation and independent confirmation is, however, still needed, pending the availability of sufficient human samples for testing between different laboratories.