Establishment of a novel capture sequencing method for patients at high risk for central nervous system infection

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Clinical Challenge - Central Nervous System (CNS) Infections in Vulnerable Patients: CNS infections are among the most devastating clinical entities, often leading to high morbidity and mortality—particularly in immunocompromised and other vulnerable patient populations. Viral encephalitis and meningitis are of particular concern, as their clinical manifestations are frequently nonspecific and conventional diagnostic methods often fail to identify the causative infectious agent. As a result, many patients receive ineffective therapies, delaying targeted management and contributing to poor outcomes.

In immunocompromised individuals, the range of infectious agents capable of infecting the CNS is markedly expanded. Viruses, bacteria, and fungi that normally do not affect immunocompetent patients, can infect the CNS and cause severe and atypical symptoms. Consequently, this expanded spectrum of pathogens is requiring simultaneous testing for numerous infectious agents. However, current diagnostic approaches—even advanced multiplex PCR panel assays—are restricted to predefined targets and remain time-consuming, resource-intensive, and incomplete. Testing comprehensively for all possible agents is impractical, leading to delayed or missed identification of causative pathogens.

Recent therapeutic advances in oncology have further increased the complexity of CNS infection diagnostics. **Novel immune-targeting treatments,** such as B-cell maturation antigen (BCMA)–targeting bispecific antibodies**, profoundly alter humoral immunity** through B-cell depletion and impaired antibody production. These therapies are associated with **markedly elevated infection rates**, including opportunistic CNS infections,. This evolving clinical context underscores the urgent need for diagnostic tools that can detect both known and emerging pathogens—even in patients with modulated immune systems or low pathogen loads.

Current Diagnostic Limitations and Preliminary Work: Metagenomic next-generation sequencing (mNGS) has emerged as a promising, culture-independent technology that enables unbiased pathogen identification without prior assumptions. Our institute has successfully demonstrated its diagnostic potential in previously unexplained cases of encephalitis and meningitis, confirming its ability to uncover rare or unexpected pathogens. However, the broad clinical adoption of metagenomics remains limited by several factors:

- Low sensitivity, e.g., in tissue, due to the high proportion of host-derived nucleic acids;
- Lack of standardized analytical validation and regulatory conformity IVDR;
- High cost and computational complexity, restricting use in routine diagnostic settings.

We propose a **high-sensitivity viral capture sequencing platform** to improve CNS infection diagnostics in immunocompromised patients. By enriching viral nucleic acids prior to sequencing, the method enhances sensitivity and reduces host background. This **scalable, cost-effective approach** bridges research and clinical diagnostics, enabling rapid and accurate detection of known and emerging viral pathogens.

Objectives:

This project aims to establish and validate a **highly sensitive capture sequencing panel and workflow** for the detection of **emerging and opportunistic viral infections in vulnerable patients with suspected CNS involvement**.

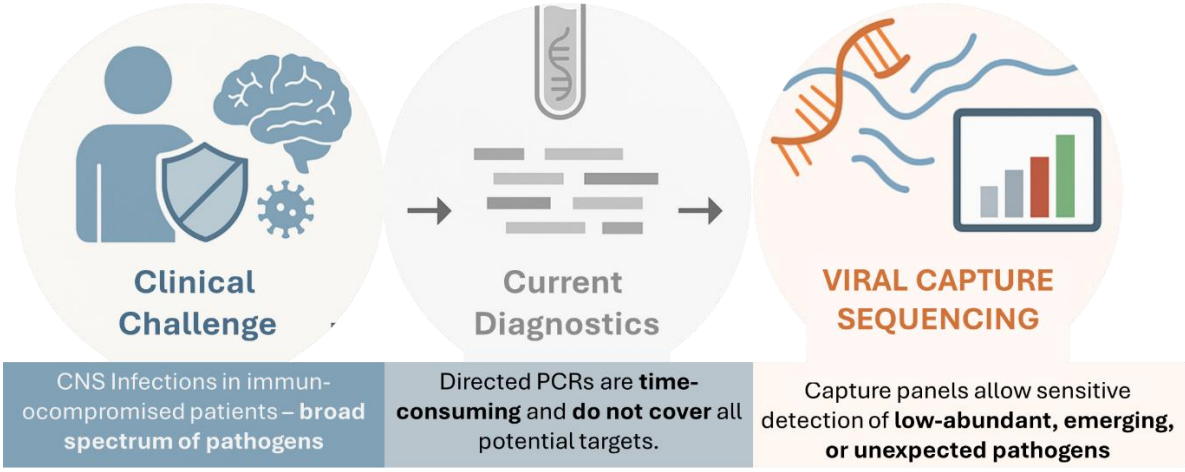
1. **Development and optimization of a viral capture sequencing workflow**

Design and evaluate capture probe panels covering a broad spectrum of clinically relevant and emerging neurotropic viruses. Optimize library preparation and enrichment protocols for low-input and host-dominated samples (e.g., CSF, biopsy material).

2. **Analytical and clinical validation**

Assess analytical sensitivity, specificity, and reproducibility using defined viral standards and clinical samples. Compare performance against conventional diagnostic methods (multiplex-

PCR, unbiased RNASeq (mNGS)) across diverse diagnostic specimen (low and high complexity samples).



Workpackages:

WP1: Development and Optimization of a Custom Viral Capture Sequencing Workflow

A custom viral capture panel will be designed to target a broad range of established and emerging CNS pathogens. Unlike large commercial panels (e.g., TWIST Viral Comprehensive Panel, >1 million probes), our design will focus on optimized coverage, reduced redundancy, and adaptability to incorporate newly discovered viral sequences. The workflow will include optimized library preparation protocols for low-input, host-rich samples such as CSF and brain tissue. Advanced bioinformatic tools will be implemented for read mapping, contamination control, genome reconstruction, and variant analysis.

WP2: Analytical Validation of Capture Sequencing Approaches

The custom panel will undergo rigorous validation against the commercial TWIST panel to evaluate performance, sensitivity, and cost-effectiveness. Using defined viral standards and well-characterized clinical samples, we will assess key metrics, including limit of detection, genome coverage, reproducibility, and accuracy across varying viral loads and sample types. Benchmarking against conventional diagnostics (targeted PCR and mNGS) will quantify relative improvements in sensitivity, breadth of detection, and turnaround time, establishing the platform's translational potential for clinical routine implementation.

Through this **systematic comparison**, the project aims to define a validated, cost-efficient capture sequencing strategy suitable for **future implementation in clinical CNS infection diagnostics**. The platform will also provide the **foundation for integration into the Netzwerk der Universitätsmedizin (NUM) study network** to establish a multicenter validation study, facilitating broader clinical implementation across German university hospitals

Project-related publications:

1. Olearo, F., M. Christner, M. **Lutgehetmann, M. Aepfelbacher, N. Fischer**, and H. Rohde. **2025**. 'Revisiting diagnostics: microbial cell-free DNA-sequencing: addressing unmet challenges in implant related cardiovascular infections', ***Clin Microbiol Infect***, 31: 510-12.
2. Sake, S. M., …, **N. Fischer,** … T. F. Schulz, T. Krey, S. Haid, and T. Pietschmann. **2024**. 'Drugrepurposing screen identifies lonafarnib as respiratory syncytial virus fusion protein inhibitor', ***Nat Commun*****,** 15: 1173.
3. Tang, H. T., …, **N. Fischer, M. Aepfelbacher,** H. Rohde, and **M. Lutgehetmann**. **2024**. 'Analytical and clinical validation of a novel, laboratory-developed, modular multiplex-PCR panel for fully automated high-throughput detection of 16 respiratory viruses', ***J Clin Virol*****,** 173: 105693.
4.Czech-Sioli, M., …**M. Lutgehetmann,** …, **M. Aepfelbacher,** … **N. Fischer. 2023**. 'Integration of Sequencing and Epidemiologic Data for Surveillance of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-Infections in a Tertiary-Care Hospital', ***Clin Infect Dis***, 76: e263-e73.
5. Heyer, A., …, **M. Lutgehetmann**, …, **M. Aepfelbacher**, J. Schulze Zur Wiesch, **N. Fischer*,** and A.Grundhoff*. **2022**. 'Remdesivir-induced emergence of SARS-CoV2 variants in patients with prolonged infection', ***Cell Rep Med*****,** 3: 100735.