Supplemental Material

Study population.

Stanford Hospital and Lucile Packard Children's Hospital are 613-bed adult and 364-bed pediatric, respectively, tertiary care academic hospitals with affiliated community clinics and extensive programs for cancer care and hematopoietic stem cell and solid organ transplant. Patients from all ordering locations (emergency department, inpatient, and outpatient) were included in this study. In addition, patients whose CSF samples were referred from outside institutions were included. Electronic medical records (EMR) were reviewed to investigate patient demographic information, CSF parameters, and results of conventional infectious disease testing performed concurrently. A subset of records in whom discordant results were obtained was further interrogated for clinical information necessary to adjudicate the infectious disease diagnosis and associated treatment decisions.

FilmArray ME Panel.

Orders were placed electronically by providers. CSF samples were tested 24 hours, 7 days a week shortly upon arrival in the laboratory, which is located 3 miles away from the hospitals. Sample processing for the FilmArray ME Panel was performed in a dedicated class II biosafety cabinet. Testing was performed per the package insert. Only one CSF sample was processed at a time with in-between surface cleaning with a bleach wipe. Four BioFire modules in random order were used for testing during the study period.

Conventional testing.

All tests were performed at Stanford Health Care Clinical Microbiology and Virology Laboratories. Cryptococcal Antigen (CrAg) Lateral Flow Assay (IMMY, Norman, OK) was performed on CSF per the package insert. For microbiological culture, when CSF volume was 1 mL or higher, samples were pre-concentrated via sedimentation at 1500 x*g* for 15 min. CSF was inoculated on blood and chocolate agars (Becton Dickinson, Durham, NC) and incubated at 37°C for 5 days. Isolates were identified with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) (Bruker, Billerica, MA). Targeted bacterial 16S rRNA and fungal ITS2/D2 sequencing was performed as previously described[8]. Viral nucleic acid amplification assays for CMV, HSV-1/HSV-2, VZV, and HHV-6 were performed at the Stanford Health Care Clinical Virology Laboratory. Testing is performed 2 to 3 times per week depending on test volume. On demand testing is available upon request for neonatal HSV evaluation. For CMV, total nucleic acids were extracted from 400µL CSF using the Pathogen Complex 400 protocol on the QiaSymphony SP (Qiagen, Germantown, MD), as previously described[9]. For HSV-1/HSV-2, VZV, and HHV-6 total nucleic acids were extracted from 200 µL (HSV-1/HSV-2, VZV) or 400 µL (HHV-6) using the EZ1 Virus Mini Kit v2.0 on the EZ1 instrument (Qiagen, Germantown, MD), according to the manufacturer's instructions. CMV, HSV-1/HSV-2, and VZV DNA detection was carried out using *artus* RGQ MDx reagents for CMV, and *artus* Analyte Specific Reagents for HSV-1/HSV-2 and VZV (all from Qiagen, Germantown, MD). CMV reactions were set up using the QiaSymphony AS module and were composed of 10 µL purified nucleic acids, 12.5 µL of CMV RG Master, and 2.5 µL of Mg-Sol. HSV-1/HSV-2 and VZV reactions were set up manually and were composed of 10 µL purified nucleic acids, 11.0 µL

of Master mix, 1.5 µL of primer/probe mix, and 2.5 µL of Mg-Sol. All reactions were run on the Rotor-Gene Q real-time PCR instrument (Qiagen, Germantown, MD). The reactions underwent 10 min at 95°C, then 10 cycles of touchdown PCR with the annealing step starting at 65°C for 30 s and decreasing by 1°C each cycle. Denaturation was at 95°C for 15 s and extension at 72°C for 20 s. Touchdown PCR was followed by 35 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 20 s. HHV-6 PCR was performed as previously described[10]. EV RT-PCR was performed using Xpert EV (Cepheid, Sunnyvale, CA) according to the manufacturer's recommendations.

Acyclovir de-escalation.

Acyclovir treatment duration was compared in patients with suspected HSV encephalitis who tested negative for HSV before (May 2016 to December 2016) and after (February 2017 to March 2019) the implementation of the FilmArray ME Panel. Treatment duration was defined as the time elapsed (in hours) between initiation of the first dose and initiation of the last dose of acyclovir. Inclusion criteria included those over the age of 18 who received more than one dose of acyclovir, with a hospital unit, including emergency department, designated as the collection department for the lumbar puncture. Given that the decision to discontinue acyclovir treatment in routine practice is frequently based on the initial CSF profile rather than the results of the FilmArray ME Panel, patients who received a single dose of acyclovir were excluded.

Clinical adjudication.

Two investigators with neurology expertise (SD, CG) performed independent chart review of discordant results (initially positive Film Array ME Panel test and negative conventional test) to determine the likelihood of true infection. Conventional test results were used as the reference method. Chart review was also performed on patients whose Film Array ME panel order was cancelled due to lack of CSF pleocytosis but conventional test was positive for ME Panel targets to determine if cancellation of the test led to treatment delays for ME Panel targets detected by conventional assays. This analysis assumes the Film Array ME Panel would have detected targets detected by conventional assays. Treatment delay was defined as initiation or escalation of antimicrobial therapy based on the timing of the conventional testing result.

Statistical analyses.

95% confidence intervals were calculated using the exact binomial (Clopper-Pearson) formula.

Supplementary Tables and Figures

Table S1. Target-specific PCR viral detection assays

Viral Target	Extraction	Real-time PCR Assay	Amplification
HSV-1/HSV-2	Qiagen EZ1	*artus* HSV-1/HSV-2	Rotor-Gene Q
VZV	Qiagen EZ1	*artus* VZV	Rotor-Gene Q

CMV	Qiagen QiaSymphony	*artus CMV*	Rotor-Gene Q
HHV-6	Qiagen EZ1	LDT	Rotor-Gene Q
Enterovirus	GeneXpert	Cepheid Xpert EV	GeneXpert

LDT, lab developed test

Figure S1. Revised FilmArray ME Panel testing and reporting algorithm for positive targets. In this algorithm selective repeat testing is performed only on positive non-viral targets. Positive viral results would not undergo repeat testing.