







# Performance Evaluation of RPIP: A Next-Generation Sequencing Platform for Pathogen Detection in Cerebrospinal Fluid

Chia-Hua Chou

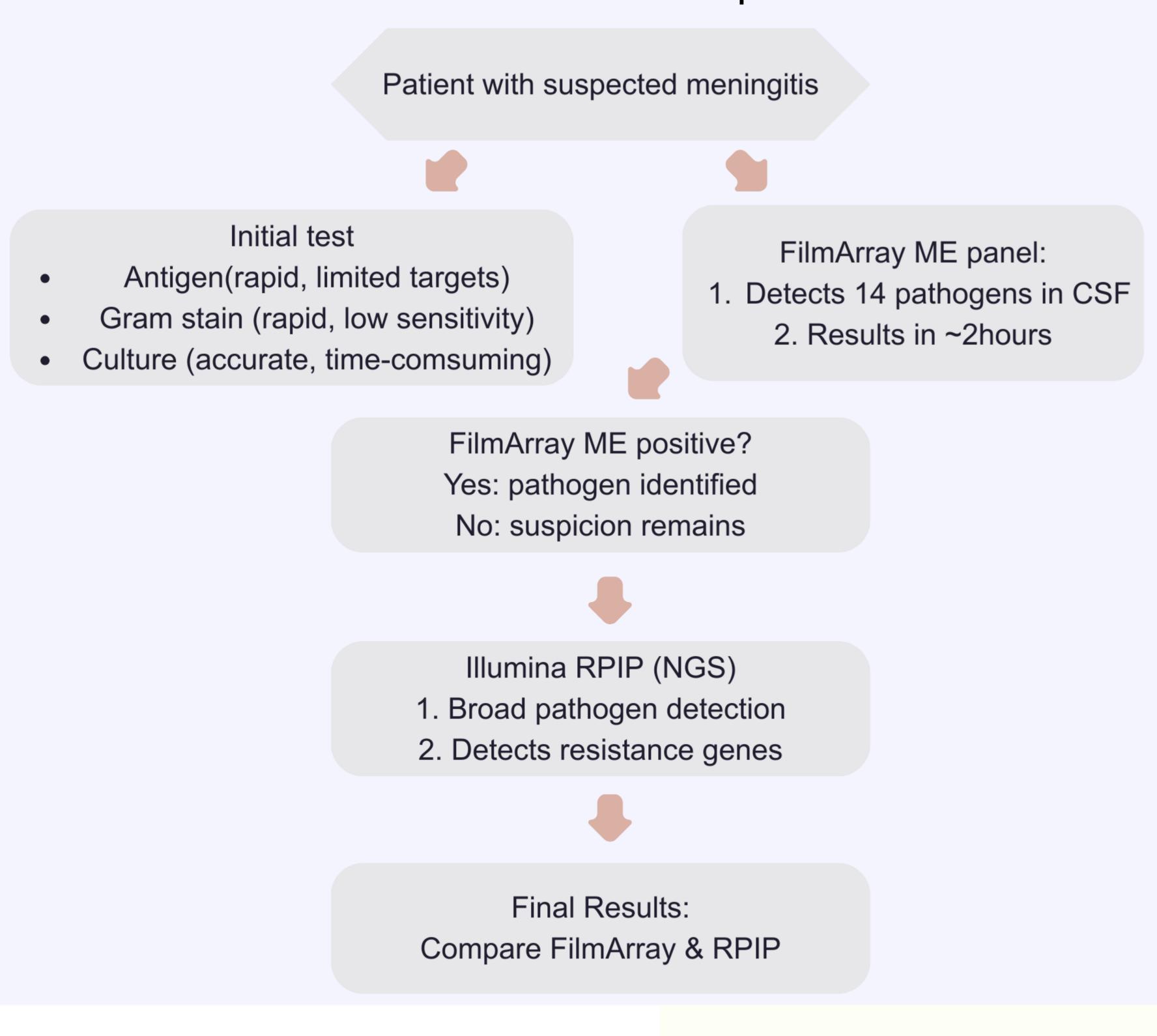
Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

### Introduction

Meningitis is a serious inflammation of the brain and spinal cord membranes, typically caused by infection. Conventional diagnostic tools such as Gram stain offer rapid results but have limited sensitivity, while culture methods are more accurate yet time-consuming. At our hospital, we use FilmArray Meningitis/Encephalitis (ME) panel, a rapid molecular diagnostic assay that identifies 14 pathogens in cerebrospinal fluid (CSF) within 2 hours. When FilmArray ME results are negative but clinical suspicion remains high, we perform additional testing using the Illumina RPIP panel—a next-generation sequencing (NGS) platform that detects a broader range of pathogens and antimicrobial resistance genes from minimal sample volumes.

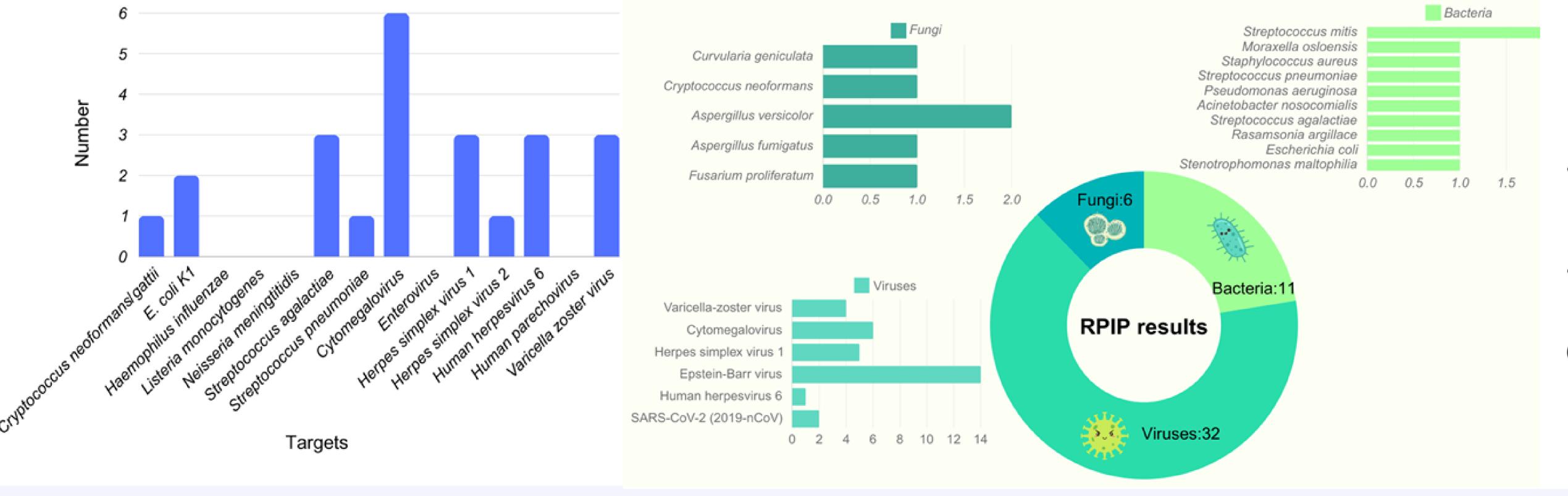
# **Material and Methods**

In this study, 104 residual CSF samples previously tested with FilmArray ME were analyzed using the Illumina RPIP and results were compared.



#### Results

FilmArray ME yielded 23 positive results, each identifying a single pathogen(Figure1.). By contrast, the RPIP assay detected 33 positive cases, comprising 11 bacterial species, 32 viral species, and 6 fungal species (Figure2). Among these, 22 samples contained a single pathogen, 7 samples had two pathogens, 3 samples had three pathogens, and 1 sample contained four pathogens (Figure3). Comparative analysis showed: 7 samples had identical results, 7 had partial overlap with additional RPIP findings, 7 were positive only by FilmArray ME, 17 only by RPIP, 2 had discordant targets, and 64 were negative by both. Overall concordance was 75% (78/104).



Solution of the second second

Figure 1. Distribution of positive results detected by FilmArray ME in 104 specimens

Figure 2. Distribution of 33 types of positive results detected by RPIP

Figure 3. Number of specimens with positive targets detected by RPIP in 104 specimens

## Conclusion

While RPIP offers broader pathogen detection through NGS, its multiple amplification steps may increase the risk of noise, especially in low-cell CSF samples. Therefore, RPIP results should be interpreted with caution and validated with clinical context and additional data analysis.