Criteria for a Positive mNGS Result

1. Bacteria (mycobacteria excluded), virus and parasites: according to Langelier's study, mNGS identified a microbe as confirmed pathogens when the clinical testing identified the microbe too. Microbes were considered potential pathogens when literature have reported the pathogenicity or the score was as least two-fold greater than of any other microbe of the same type identified in the patient[1].

2. Fungi: mNGS identified a microbe (species level) whose coverage rate scored 5-fold higher than that of any other fungus because of low absolute abundance in many samples and the contaminating fungal DNA[2, 3].

3. Mycobacteria: Mycobacterium tuberculosis (MTB) was considered positive when at least 1 read was mapped to either the species or genus level due to the Mycobacteria cell wall difficult to disruption to lyse the organism for nucleic acid release and low possibility for contaminationqqsx[4, 5]. The efficient extraction methods are critical to achieve unbiased sequencing. Nontuberculous mycobacteria (NTM) were defined as positive when the mapping read number (genus or species level) was in the top 10 in the bacteria list due to the balance of hospital-to-laboratory environmental contamination and low yield rate[6, 7].

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