

Supplementary Table S1:

Part 1: Basic Information of the Expert

- 1 Your Name:
- 2 Your Gender: Male Female
- 3 You have been using metagenomic next-generation sequencing (mNGS) technology for the diagnosis of infectious eye diseases for _____ years.

Part 2: Expert Consensus Evaluation Items

Questionnaire Items

I. Application and Indications

1. mNGS can be used to assist in the etiological diagnosis of suspected infectious eye diseases, including infectious keratitis, endogenous endophthalmitis, and exogenous endophthalmitis.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

2. For suspected acute suppurative keratitis (bacterial, fungal, amoebic infections) and acute infectious endophthalmitis (bacterial, fungal infections), due to the rapid progression of the disease and the significant risk of visual loss, samples can be collected and sent for mNGS immediately to identify the pathogenic microorganisms.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

3. For suspected ocular herpes virus infections and ocular parasitic infections, quantitative polymerase chain reaction (qPCR) or antibody detection methods can be prioritized based on

disease characteristics and diagnostic direction, and mNGS should not be the first choice.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

4. For other types of ocular inflammation, such as conjunctivitis, blepharitis, and dacryocystitis, if traditional microbiological tests are negative and empirical treatment has been ineffective, mNGS can be considered if an infectious disease is still highly suspected.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

5. For patients with primary immunodeficiency, granulocytopenia, AIDS, and those receiving immunosuppressive therapy, due to the wide variety of potential pathogens, including rare pathogens and mixed infections, mNGS can be performed on the first sample submission.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

6. For clinically suspected ocular infectious diseases, DNA sequencing is generally recommended. If the patient has a history of travel to endemic areas or a history of systemic RNA virus infection, and the ocular manifestations are highly suggestive of an infectious disease, RNA sequencing can also be considered.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

II. Sample Selection, Collection, Management, and Transport

7. Samples should be collected as close as possible to the site of infection based on the patient's specific ocular signs and surgical indications.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

8. Before sample collection, patient or guardian should be informed about the relevant content of mNGS, including the purpose of the test, positive rate, necessity, limitations, cost, expected reporting time, disposition of remaining samples, storage duration, whether remaining samples can be anonymized for research projects, and other optional testing items.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

9. The testing intent should be clearly defined and communicated to the laboratory. After sample collection, following items should be verified: patient basic information, sampling date, sample type, name of the hospital, and whether the testing demand are consistent with the actual requirements.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

10. After standard collection, ocular samples intended for DNA sequencing can be stored at -20°C for no more than 7 days, while samples intended for RNA sequencing should be stored at -80°C.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

11. Ocular samples should be transported to the laboratory at low temperatures. Samples expected to be transported within 24 hours can be transported with ice packs, while samples expected to be transported within 24-72 hours should be transported with dry ice. For RNA sequencing, ribonuclease (RNase) inhibitors can be added in proportion.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

12. In cases of keratitis or corneal ulcer, if mNGS and other pathogen microbiological laboratory tests (KOH wet mount, Gram staining, bacterial culture, fungal culture, Giemsa staining, fluorescent staining) are to be performed simultaneously, the overall microbial detection rate of mNGS is not affected by the order of sample collection.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

III. Laboratory Testing, Reporting, and Quality Control

13. The laboratory should establish pre-processing procedures for different samples, develop nucleic acid extraction methods for samples with extremely low nucleic acid content, define standards for nucleic acid quality and library output, and determine the minimum sequencing data volume required for pathogen detection.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

14. Each batch of experiments should include internal references, negative controls, and positive controls.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

15. An analysis pipeline suitable for ocular infectious diseases should be established by combining internationally recognized data quality control software, alignment software, and species analysis software, known outcome samples and quality control samples should be used for bioinformatics

analysis simulation testing.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

16. The mNGS report should include the following information: patient information, sample information, testing method, testing scope, results, testing institution, reporter information, total sequencing reads, total reads after quality control, list of detected pathogens, number of specific sequences for detected pathogens, testing scope, sequencing data quality, technical description of the test, sensitivity and specificity of the testing method, and limitations.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

17. Standard operating procedures (SOPs) for the entire mNGS process should be established, including sampling requirements, sample processing, nucleic acid extraction, library preparation, sequencing, and bioinformatics analysis. If the reagents, analysis software, parameters, or databases used in the process change, partial or full revalidation should be performed.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

IV. Clinical Interpretation of Reports

18. Due to differences in sequencing platforms, varying genome lengths of different microorganisms, and varying severity of infections among patients, it is not possible to establish a unified positive/negative interpretation standard for all microorganisms as pathogenic agents.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

19. If the microorganism detected in the mNGS has clear ocular pathogenicity, and the patient's medical history and ocular signs are consistent with the characteristics of ocular diseases caused by the detected microorganism as reported in the published literature, it can be preliminarily identified as the responsible pathogens.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

20. If the reported microorganism is consistent with the results of other traditional microbiological tests performed on the same sample, or with the results of other systemic tests performed on the patient, it can be determined as the responsible pathogens.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

21. When common human colonizing bacteria and environmental bacteria

(e.g., *Propionibacterium acnes*, *Moraxella osloensis*, *Acinetobacter junii*, *Malassezia restricta*)

were detected by mNGS with high sequence counts and relative abundance, and they dominate compared to other detected microorganisms, their pathogenic potential should be considered in light of previous reports and the patient's clinical features.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

22. When non-human colonizing bacteria (e.g., *Talaromyces marneffeii*, *Acanthamoeba*, *Rickettsia*)

were detected by mNGS, which are uncommon in the environment, their pathogenic potential should be considered in light of the patient's clinical features.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

23. When intracellular bacteria (e.g., *Mycobacterium tuberculosis*, *Brucella*, *Bartonella henselae*) and fungi were detected by mNGS, even if the sequence counts for these microorganisms in the report are low, their pathogenic potential should be considered in light of the patient's clinical manifestations and other auxiliary test results.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

24. If Epstein-Barr virus (EBV) was detected by mNGS, it does not necessarily indicate that EBV is the responsible pathogen for the patient's eye disease.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

25. Currently, there is no evidence to suggest a clear correlation between the sequence counts detected by mNGS and the absolute load of pathogens. Therefore, sequence counts should not be used as an indicator to judge the severity of infection or prognosis when interpreting mNGS results.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

26. If the mNGS report is negative, it cannot be used as a basis to completely rule out ocular infectious diseases. Factors such as improper sample collection, transport, and storage leading to false negatives should be excluded, and microorganisms below the detection threshold should be considered for further judgment.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

27. If DNA test results are negative, the possibility of an RNA virus infection causing the eye disease should be considered.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

28. If the mNGS report is negative, the possibility of non-infectious ocular diseases, including autoimmune uveitis, masquerade syndromes, and retinal vascular diseases, should be considered.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

29. Since mNGS has relatively low detection efficiency for certain pathogens (e.g., *Mycobacterium tuberculosis*, fungi), if infection with these pathogens is suspected, it is recommended to combine systemic serological testing, imaging, and other testing methods on the same sample for comprehensive clinical evaluation.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

v. Clinical Measures Based on Report Results

30. If the microorganisms detected by mNGS are highly suggestive of being the responsible pathogens, targeted anti-infective treatment should be initiated based on the clinical situation and the species of the microorganism.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

31. If the microorganisms detected by mNGS are rare and the clinical significance as responsible

pathogens cannot be confirmed, other test results and previous published literature should be considered for joint judgment, and diagnostic treatment may be necessary.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

32. If a newly emerging pathogen is detected by mNGS, it should be verified using other laboratory testing methods, and a report to the Chinese Center for Disease Control and Prevention (China CDC) or other relevant authorities will be necessary.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

33. If the mNGS result is negative, but the clinical presentation is highly suggestive of infection and empirical anti-infective treatment is effective, it is recommended to continue the current treatment and not to terminate effective anti-infective therapy based on the negative mNGS result.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

34. If the mNGS report indicates the detection of drug resistance genes, the results have limitations and should not be solely relied upon for selecting anti-infective drugs.

1. Strongly disagree 2. Disagree 3. Uncertain 4. Agree 5. Strongly agree

Part 3: Expert Familiarity and Evaluation Basis

1. Overall familiarity with all items in the expert consultation form

1 Very unfamiliar

2 Somewhat unfamiliar

□ 3 Neutral

□ 4 Somewhat familiar

□ 5 Very familiar

Definition of familiarity

Expertise level	Defintion	Qualification on Criteria
Very familiar	Processes both profound theoretical knowledge and extensive practical experience	<ol style="list-style-type: none">1. Independently performed or participated in ≥ 100 mNGS tests of ocular samples in past 5 years2. Authored or contributed to relevant guidelines or consensus statements of other samples
Somewhat familiar	Commands core concepts with demonstrated practical experience	<ol style="list-style-type: none">1. Participated in ≥ 50 mNGS tests of ocular samples in past 5 years2. Authored or contributed to original research of the application of mNGS in ocular infection
Neural	Understands fundamental concepts with several hands-on experience	<ol style="list-style-type: none">1. Participated in ≥ 10 mNGS tests of ocular samples in past 5 years2. Capable of independent report interpretation and clinical guidance
Somewhat unfamiliar	Familiar with basic terminology only	<ol style="list-style-type: none">1. Engaged in limited case discussion2. Knowledge acquired solely through

literature

Very unfamiliar

No relevant knowledge or experience

No prior exposure to mNGS technology

2. The degree to which the following judgment criteria influenced your decision-making

Judgement criteria	High	Moderate	Low
Practical experience			
Fundamental theory			
Peer opinions			
Intuitive perception			
Clinical literature			

Supplementary Table S2. Consensus on mNGS application across the clinical diagnosis-treatment pathway

Clinical Stage	Summary of Consensus Points	Relevant Questionnaire Items
<p>Clinical Application & Testing Decision <i>(Determining if and when to use mNGS)</i></p>	<ul style="list-style-type: none"> • mNGS is applicable for the etiological diagnosis of suspected ocular infectious diseases (e.g., keratitis, endophthalmitis). • mNGS testing should be initiated promptly for acute, severe infections, but must be performed alongside conventional microbiological tests. • Testing can be considered for the first sample submission in immunocompromised patients to cover a broad spectrum of pathogens. • mNGS is not the first-line test for suspected herpesvirus or parasitic infections. • Sampling from the correct anatomical site, along with standardized sample transport and storage, is critical. 	<p>1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11</p>
<p>Result Interpretation & Diagnostic Integration <i>(Integrating mNGS results into clinical diagnosis)</i></p>	<ul style="list-style-type: none"> • mNGS is a qualitative tool; sequence counts do not correlate with absolute pathogen load or infection severity. • Positive results must be interpreted in the context of the patient's history, ocular signs, imaging, and other laboratory findings. • Caution is required when interpreting results indicating commensals, environmental 	<p>18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29</p>

contaminants, intracellular bacteria/fungi, or viruses like EBV.

- A negative result cannot completely rule out infection; technical factors should be excluded, and non-infectious etiologies considered.
- The possibility of an RNA virus infection should be considered if DNA-based mNGS is negative.
- Targeted anti-infective therapy can be initiated if the detected microorganism is highly suggestive of being the causative pathogen.

Treatment Decision & Clinical Management

(Guiding management based on mNGS reports)

- For rare or novel pathogens detected, results should be verified by alternative methods before guiding definitive therapy.
- Antimicrobial resistance genes reported by mNGS have limitations and should not be the sole basis for drug selection.
- Effective empirical anti-infective therapy should not be discontinued solely based on a negative mNGS result if clinical suspicion remains high and the patient is responding.

30, 31, 32, 33, 34
