Letter

Chinese Expert Consensus on the Application of Metagenomic Sequencing Technology in Ocular Infectious Diseases: A Delphi Method

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Metagenomic next-generation sequencing (mNGS) is a culture-independent technique that directly extracts and sequences all nucleic acids from clinical specimens. By leveraging high-throughput sequencing and bioinformatic analysis, it characterizes the entire microbial landscape, including bacteria, fungi, viruses, and parasites. This approach significantly broadens detection coverage, improves sensitivity, and reduces turnaround time compared with conventional diagnostic methods. Since its initial application to suspected infectious uveitis by Doan et al. in 2006, mNGS has been increasingly integrated into ophthalmic practice to facilitate pathogen identification or exclusion[1].

The complexity of the mNGS workflow—from indication selection and specimen collection to laboratory testing and clinical interpretation—necessitates standardized guidelines for its application into ophthalmic infectious diseases. To address this need, the Ophthalmology Testing and Inspection Group (OTIG) conducted a Delphi survey among ophthalmologists and microbiologists. The survey aimed to evaluate current practices throughout the testing process and to develop a unified consensus to guide the standardized use of mNGS in ophthalmology. This consensus has been registered with the Practice Guideline Registration for Transparency Platform (PREPARE-2024CN965).

This study employed the Delphi method—a classical approach for achieving expert consensus widely utilized in healthcare research, including medical technology assessment and clinical management. This method enables a large panel of experts to be consulted via structured questionnaires and allows for the clarification and resolution of ambiguities in questionnaire wording[2]. The Delphi method used in this study comprised four rounds as follows:

Round 1: A Delphi expression of opinions was developed by OTIG and was formulated into a questionnaire.

Round 2: Experts were invited by OTIG to complete the questionnaire.

Round 3: Participants rated their level of agreement with each statement in the questionnaire.

Round 4: The results of the ratings were analyzed and summarized.

Sixty experts (18 ophthalmologists and 42 clinical laboratory specialists) from mainland China were invited based on their experience with mNGS applications, management of infectious eye diseases, participation in academic conferences, and publication records. The questionnaire items were developed and finalized by the principal authors (YT and STX), and pilot-tested by another author (ZYQ).

The 34-item questionnaire encompassed five key domains of mNGS: 'Application and Indications', 'Sample Selection, Collection, Management, and Transport', 'Laboratory Testing, Reporting, and Quality Control', 'Clinical Interpretation of Reports', and 'Clinical Measures Based on Report Results'. It was administered via an online platform, and all 60 experts participated anonymously. A five-point Likert scale (1 = strongly disagree; 2 = disagree; 3 = uncertain; 4 = agree; 5 = strongly agree) was used to evaluate each participant's level of agreement with each questionnaire item. Items for which the combined percentage of '4 + 5' responses exceeded 80% were considered to have reached consensus.

In addition to mNGS-related content, the questionnaire also collected information on the

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experts' years of experience applying mNGS in ophthalmic infectious diseases and their overall familiarity with the questionnaire items (1 = very unfamiliar, 2 = somewhat unfamiliar, 3 = neutral, 4 = somewhat familiar, 5 = very familiar). Participants were also asked to indicate the primary basis for their judgments, including 'Practical experience', 'Fundamental theory', 'Peer opinions', 'Intuitive perception' and 'Clinical literature', ranked from 'High' to 'Low'. The complete questionnaire is presented in Supplementary Table S1.

Before initiating the Delphi survey, an online expert meeting was held to discuss the clinical background, current applications, and major controversies surrounding the use of mNGS in ocular infections. Following this meeting, the questionnaire was distributed electronically. Upon completion of data collection, YT, STX, and ZYQ independently analyzed the responses to determine consensus levels.

All 60 invited experts responded to the questionnaire items. The median number of years for which the experts had applied mNGS to ophthalmic infectious diseases was 5 years (interquartile range: 5–5.5 years). The proportions of experts who reported being neutral, somewhat familiar, and very familiar with the questionnaire items were 11.7%, 53.3%, and 35%, respectively. Table 1 illustrates the extent to which different evaluation criteria influenced the invited experts.

Among all questionnaire items, the average score on the Likert scale was 4.6 points. The proportion of items with expert votes of '4 + 5' was 96.0%, with '4' votes accounting for 27.9% and '5' votes accounting for 68.1%. For item 12, the '4 + 5' proportion was 68.3%, which did not meet the consensus threshold, while all other items achieved consensus. Table 2 presents the detailed results of expert voting. Table 3 shows the consensus-formation status of the questionnaire items. To clearly illustrate the key application nodes of mNGS throughout the clinical diagnosis-treatment pathway, we mapped expert consensus statements to their respective clinical stages based on the standard clinical workflow (Supplementary Table S2).

Since its introduction in 2016, mNGS has been increasingly used for etiological diagnosis of suspected ocular infections. Cohort studies and case reports have demonstrated its ability to detect pathogens in various ophthalmic infections, with positive detection rates of 88.9% and 83.9% for endophthalmitis and keratitis, respectively[3,4]. A large prospective study further reported 87.8% sensitivity and 77.3% concordance for intraocular fluid testing[5]. These findings support the use of mNGS as a valuable tool for diagnosing infectious eye diseases. To standardize its application, we conducted this Delphi study, achieving consensus on 33 of the 34 items.

All six items in the 'Application and Indications' section achieved consensus (91.7%–98.3% agreement), focusing on case selection and optimal timing for mNGS testing. Experts recommended prompt mNGS for acute ocular infections (e.g., suppurative keratitis and infectious endophthalmitis), but emphasized that it should be performed in conjunction with conventional tests. For suspected corneal infections, concurrent corneal scraping cytology, culture, and confocal microscopy are recommended; for suspected intraocular infections, aqueous or vitreous fluid should similarly undergo cytology, culture, and other molecular tests[6]. Supporting evidence indicates that mNGS exhibits a higher bacterial detection rate than other methods in keratitis, with fungal detection comparable to confocal microscopy[3], and while mNGS outperforms culture in endophthalmitis, culture remains a diagnostic gold standard[4]. Notably, mNGS cannot detect *Toxocara* in ocular toxocariasis due to the absence of DNA in intraocular fluid, making serological testing the preferred diagnostic approach[7].

Five of six items in the 'Sample Selection, Collection, Management, and Transport' section reached consensus (91.7%–100% agreement). The only item without consensus (68.3% agreement) concerned whether the sequence of corneal sampling affects mNGS detection rates. Although Redd et al. reported consistent detection rates regardless of order when mNGS was performed

Table 1. Influence of different judgment criteria on the invited experts

Influence(Number/Percentage)	Practical experience	Fundamental theory	Peer opinions	Intuitive perception	Clinical literature
High	44 (73.3%)	39 (65.0%)	18 (30.0%)	4 (6.6%)	28 (46.7%)
Moderate	15 (25.0%)	20 (33.3%)	33 (55.0%)	28 (46.7%)	32 (53.3%)
Low	1 (1.7%)	1 (1.7%)	9 (15.0%)	28 (46.7%)	0 (0%)

alongside other methods (e.g., KOH mount, Gram stain, culture)[8], collecting the mNGS sample last may increase discordance, possibly due to contamination. This divergence highlights limited practical experience, underscoring the need to individualize sampling sequence while ensuring accurate lesion targeting. The consensus emphasized that in situ sampling is critical, as selecting an inappropriate sample type (corneal tissue, aqueous humor, or vitreous) for a given infection can lead to false-negatives[9]. Samples should be obtained as close as possible to the infection site. For example, superficial epithelial lesions should be scraped, while deep stromal or endothelial lesions may require

Table 2. Detailed results of the expert voting

Item	Mean	No. score 4	No. score 5	% Score 4	% Score 5	Interquartile range
1	4.78	10	49	16.7	81.7	0
2	4.60	15	42	25.0	70.0	1
3	4.32	24	31	40.0	51.7	1
4	4.70	12	45	20.0	75.0	0
5	4.62	20	39	33.3	65.0	1
6	4.63	17	41	28.3	68.3	1
7	4.92	5	55	8.3	91.7	0
8	4.75	6	51	10.0	85.0	0
9	4.95	3	57	5.0	95.0	0
10	4.62	13	42	21.7	70.0	1
11	4.72	10	47	16.7	78.3	0
12	3.87	20	21	33.3	35.0	2
13	4.77	8	50	13.3	83.3	0
14	4.73	10	48	16.7	80.0	0
15	4.68	14	44	23.3	73.3	1
16	4.77	11	48	18.3	80.0	0
17	4.80	9	50	15.0	83.3	0
18	4.35	21	32	35.0	53.3	1
19	4.52	25	33	41.7	55.0	1
20	4.50	22	35	36.7	58.3	1
21	4.50	24	33	40.0	55.0	1
22	4.58	25	35	41.7	58.3	1
23	4.63	22	38	36.7	63.3	1
24	4.50	28	30	46.7	50.0	1
25	4.43	23	33	38.3	55.0	1
26	4.67	20	40	33.3	66.7	1
27	4.43	25	31	41.7	51.7	1
28	4.63	19	40	31.7	66.7	1
29	4.75	15	45	25.0	75.0	0
30	4.65	21	39	35.0	65.0	1
31	4.67	20	40	33.3	66.7	1
32	4.78	10	49	16.7	81.7	0
33	4.73	16	44	26.7	73.3	1
34	4.48	26	32	43.3	53.3	1

Table 3. Consensus formation of the questionnaire items

No.	Item	% Score 4+5	Final concensus
1	mNGS can be used to assist in the etiological diagnosis of suspected infectious eye diseases, including infectious keratitis, endogenous endophthalmitis, and exogenous endophthalmitis.	98.3	Yes
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.	95.0	Yes
3	For suspected ocular herpes virus infections and ocular parasitic infections, qPCR or antibody detection methods can be prioritized based on disease characteristics and diagnostic direction, and mNGS should not be the first choice.	91.7	Yes
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.	95.0	Yes
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.	98.3	Yes
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.	96.7	Yes
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.	100.0	Yes
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.	95.0	Yes
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.	100.0	Yes
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.	91.7	Yes
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.	95.0	Yes
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.	68.3	No
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.	96.7	Yes
14	Each batch of experiments should include internal references, negative controls, and positive controls.	96.7	Yes
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.	96.7	Yes
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.	98.3	Yes
17	SOP 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.	98.3	Yes

Continued

No.	Item	% Score 4+5	Final concensus
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.	88.3	Yes
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.	96.7	Yes
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.	95.08	Yes
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.	95.0	Yes
22	When non-human colonizing bacteria (e.g., *Talaromyces marneffei, 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.	100.0	Yes
23	When intracellular bacteria (e.g., *Mycobacterium uberculosis, 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.	100.0	Yes
24	If EBV was detected by mNGS, it does not necessarily indicate that EBV is the responsible pathogen for the patient's eye disease.	96.7	Yes
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.	93.3	Yes
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.	100.0	Yes
27	If DNA test results are negative, the possibility of an RNA virus infection causing the eye disease should be considered.	93.3	Yes
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.	98.3	Yes
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.	100.0	Yes
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.	100.0	Yes
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.	100.0	Yes
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.	98.3	Yes
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.	100.0	Yes
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.	96.7	Yes

Note. mNGS, metagenomic next-generation sequencing, qPCR, quantitative polymerase chain reaction, AIDS, acquired Immune Deficiency Syndrome, DNA, deoxyribonucleic acid, RNA, ribonucleic acid, SOP, standard operating procedure, EBV, Epstein-Barr virus

aqueous humor; severe anterior chamber inflammation warrants aqueous sampling, whereas predominant vitritis necessitates vitreous fluid. In cases of pan-ocular inflammation, sample choice should be guided by safety and accessibility.

All five items in the 'Laboratory Testing, Reporting, and Quality Control' section achieved consensus (96.7%–98.3% agreement). The mNGS workflow is complex, encompassing sample preprocessing, nucleic acid extraction, library construction, sequencing, data preprocessing, human sequence removal, microbial sequence alignment, microbial annotation, and report generation. Although no universal standard operating procedure exists due to inter-laboratory variations, general standards include sequencing accuracy ≥99.9% and Q30 ≥85%. For intraocular fluids, a data volume of 18 million reads yields 87.8% sensitivity[5]. Reports should specify genomic coverage and relative abundance, categorizing microorganisms by clinical relevance (e.g., high or low relevance, potential background). To aid clinical interpretation, species annotations should incorporate epidemiological and ocular pathogenicity data[10].

All 12 items in the 'Clinical Interpretation of Reports' section reached consensus (88.3%–100% agreement). Accurate interpretation is crucial for linking sequencing results to clinical diagnosis. Experts emphasized that bioinformatics findings alone cannot confirm pathogenesis; clinical context—including patient history, clinical signs, imaging, and laboratory data —remains indispensable. For instance, *Bartonella henselae* and *Brucella melitensis* identified by mNGS required serological confirmation in previous studies[11,12]. Caution is warranted given potential false positives (mNGS-culture concordance: 70%–75%)[4,13] and low sequence counts in intracellular infections (e.g., *M. tuberculosis*: 1; *A. fumigatus*: 15)[4,14]. Consensus strongly supported mNGS as a qualitative tool (93.4% agreement), noting that sequence counts vary across platforms[15], poorly reproducible (coefficient of variation ≥20%)[16,17], and unrelated to pathogen load or severity. A quality-controlled negative result may help exclude infection[18]. However, negatives in suspected intracellular or fungal cases require cautious assessment. For suspected fungal infections, multiple tests on the same sample may be necessary. Previous studies have shown that for patients with suspected fungal endophthalmitis, the clinical concordance rate of combined testing (96.2%) is higher than that of mNGS alone (86.5%) or β-D-glucan (BDG) testing alone (88.5%)[9].

All five items in the 'Clinical Measures Based on Report Results' section achieved strong consensus (96.7%–100.0% agreement), reflecting expert support for mNGS-guided treatment. However, conventional mNGS has limitations in detecting resistance and virulence genes, cannot predict gene expression, and lacks robust species-level gene classification, making it difficult to correlate genetic findings with microbial phenotypes[19]. Thus, antimicrobial selection should not rely solely on mNGS results.

In the questionnaire, experts also conducted a self-assessment to evaluate the influence of different evaluation criteria. Practical experience (73.3%) and fundamental theory (65.0%) emerged as the predominant factors exerting a 'high' degree of influence on their judgments, whereas intuitive perception had the least impact (6.6%). This indicates that the consensus was primarily grounded in evidence-based practice and scientific principles, which likely contributed to the high agreement rates. The strong correlation between experiential and theoretical foundations further enhances the reliability of the consensus.

Ophthalmic infections are often severe and can cause irreversible vision loss if not promptly diagnosed. The increasing application of mNGS has established it as an essential tool for pathogen detection. This consensus provides standardized guidance across key stages of mNGS application—from sampling to interpretation—supporting its rational clinical use. Future work should focus on personalized testing protocols, pathogen-specific thresholds, and integration with multi-omics technologies to advance precision diagnostics, emphasizing continued collaboration between clinicians and laboratory specialists[20] .

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Consent to Publish All authors confirm that the publication of this work is approved by all co-authors. This work has not been published previously, and is not under consideration for publication elsewhere. Written consent to publish this information was obtained from all the study participants.

Data Sharing All data generated or analyzed during this study are included in this published article. The supplementary materials will be available in www.besjournal.com.

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