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Validation of a multi-species-specific PCR panel to diagnose patients with suspected postoperative bacterial endophthalmitis

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ABSTRACT.

Introduction: **Currently, patients suspected of endophthalmitis are referred to a tertiary centre for a vitreous biopsy and bacterial culture, thereby causing a treatment delay for the intravitreal antibiotics injection. We developed a new diagnostic tool, multi-mono-PCR (mm-PCR), not requiring viable bacteria, allowing antibiotic injection without delay. Performance of mm-PCR was tested on biopsies from patients with suspected postoperative endophthalmitis with known bacterial culture results.**

Methods: **Most frequently occurring pathogens in endophthalmitis were determined using published data and treatment logs of endophthalmitis patient of the Rotterdam Eye Hospital.Vitreous biopsies from patients with suspected endophthalmitis were aliquoted in two parts. One part was sent out for bacterial culture and another was stored at −80°C for mm-PCR analysis and, as a backup, also by panbacterial PCR. Twelve vitreous samples from patients not suspected of having endophthalmitis were added as control samples.**

Results: **Concordancy between bacterial culture and mm-PCR was 89% (24 of 27). All twelve control samples were negative. In three nonconcordant samples, the PCR results were most likely the correct ones.**

Conclusion: **mm-PCR results are highly concordant with bacterial culture. mm-PCR with panbacterial PCR as backup could be considered a diagnostic tool in patients with endophthalmitis, which would allow us to reverse the order of diagnosis and treatment while maintaining diagnostic surveillance, thereby preventing treatment delay.**

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Introduction

Endophthalmitis is a rare but very severe eye infection, which can lead to irreversible loss of vision within hours of the onset of symptoms (Durand 2017). The cause of endophthalmitis can be exogenous, such as ocular surgeries, or endogenous, like bacteraemia or fungemia. Most cases of endophthalmitis are exogenous (Durand 2017). Risk of developing endophthalmitis is estimated at 0.008–0.092% following intravitreal injection and 0.03–0.70% following cataract surgery (Ong et al. 2019). Despite the low incidence rate, it causes a serious health problem due to the high treatment volume.

The landmark Endophthalmitis Vitrectomy Study (EVS) (Endophthalmitis Vitrectomy Study 1995) in 1995 established that intravitreal antibiotics should be used, rather than systemic antibiotics, and suggested that core vitrectomy would be better than vitreous biopsy in patients presenting with light perception only.

Currently, when a patient is being suspected of having endophthalmitis, a vitreous biopsy is taken before starting empiric treatment with intravitreal antibiotics. The biopsy is needed to detect and confirm the diagnosis of a bacterial endophthalmitis through bacterial culture. Generally, a referral to a tertiary medical hospital is required for the expertise that is needed for a vitreous biopsy, therefore causing a serious treatment delay.

Delay in treatment could potentially lead to more functional retinal loss. In a rabbit model of severe endophthalmitis by *Bacillus cereus*, Michelle C. Callegan et al. (2011) found less visual loss when treatment combined with vitrectomy and vancomycin was started at 4 hrs than 5 or 6 hrs. In Treatment after 4 hrs with vancomycin alone had the same results as additional vitrectomy. In clinical

practice, early antibiotics would be more feasible and safer than early vitrectomy for all patients with suspected endophthalmitis (van Meurs & van Dissel 2018). Such an approach is confirmed by a study on treatment of bacterial meningitis, a disease that has many similarities with bacterial endophthalmitis, in which early administration of antibiotics was correlated with a better outcome (Grindborg et al. 2015).

To overcome antibiotic treatment delay, a reliable diagnostic tool is needed that is able to detect pathogens even after intravitreal injection with antibiotics. In this way, the antibiotics could be administered immediately, before the vitreous biopsy is taken in a tertiary medical centre. Polymerase chain reaction (PCR) targeting the bacterial 16S rRNA gene, followed by DNA sequencing to identify the bacterial species, has shown to be potentially a reliable diagnostic method (Cornut et al. 2014; Pongsachareonnont, Honglertnapakul & Chatsuwan 2017; Mishra et al. 2019; Sandhu et al. 2019). Other studies have reported the use of real-time (rt) PCR—a method in which DNA amplification and detection of the target sequence occur at the same time (Bispo et al. 2011; Sugita et al. 2011; Sugita et al. 2013). Joseph et al. (Lohmann et al. 2000), included 64 patients with suspected postfako endophthalmitis, achieving a 66% identification rate for rtPCR and 34% in bacterial culture. Bispo et al. (2011) used rtPCR for universal 16S rRNA-DNA and specific probes for Gram determination, followed by sequencing, with an identification rate of 95.3 versus 53% by culture. Goldschmidt et al. (2009) used rtPCR to specifically identify pathogens to the genus level including *Staphylococcus, Streptococcus, Haemophilus, Pseudomonas, Acinetobacter, Enterobacteriaceae, Propionibacteriaceae* and *Corynebacterium*. An identification rate of 90% was achieved by rtPCR versus 60% by culture.

Only one study used rtPCR on species level for *Staphylococcus aureus* and *Streptococcus pneumoniae* and compared the results with panbacterial PCR and bacterial culture. One limitation of this study was the low prevalence of *S. aureus* and *S. pneumoniae* (Six and three cases of 153, respectively) (Kosacki et al. 2020).

However, to date, these PCR methods have only been used as a complementary tool for bacterial culture, and therefore, the administration of intravitreal antibiotics is still delayed in patients with endophthalmitis. The reluctant introduction of molecular methods in the diagnosis of endophthalmitis might be caused by the sensitivity to contaminating DNA of these methods (Ugahary et al. 2004; Bispo et al. 2011), the need for a sophisticated laboratory, and the long time required for the identification of organisms that cause endophthalmitis.

In this report, we introduce the multi-mono-PCR (mm-PCR), a method that is low cost, fast and reliable and that does not need a highly sophisticated laboratory. The mm-PCR consists of a series of 20 real-time PCRs that each separately targets one of the bacterial species that may cause endophthalmitis. In addition, the panel of the 20 separate bacterial targets contains a PCR for the 16S rRNA gene, a universal bacterial target that will detect all bacterial species, including those that were not included in the panel. The mm-PCRs are run simultaneously in a single run on a 96-well real-time PCR instrument, a procedure that can be easily implemented in any molecular diagnostic laboratory. Each of the individual PCRs of the mm-PCR panel is interpreted by the Cp value and by melting curve analysis.

The purpose of this study was to evaluate mm-PCR by comparing it with conventional bacterial culture in the diagnosis of suspected bacterial endophthalmitis. If concordancy were acceptable, it would allow us to reverse the order of biopsy and treatment while maintaining diagnostic surveillance.

Material and Methods

Study design

We performed a comparative laboratory study on vitreous biopsy samples of patients treated for suspected bacterial endophthalmitis. In the Rotterdam Eye Hospital, the use of patient's waste material is allowed when used for purposes related to the original clinical treatment. This study (OZR-2017-10) was evaluated and approved by our Institutional Review Board. The Dutch Medical Research Involving Humans Act (WMO) did not apply to this study. Written informed consent for use of data from medical records and left-over samples from diagnostics has been obtained.

Clinical samples

Undiluted vitreous biopsies were obtained from patients with suspected endophthalmitis from 2017 to 2019, with an additional twelve control vitreous samples from twelve different patients with macular hole, macular pucker, or retinal detachment. One part was immediately submitted for bacterial culture (Department of Medical Microbiology, Maasstad Hospital Rotterdam, the Netherlands), and another part was in an encoded manner stored in the Rotterdam Eye Hospital Biobank at −80°C. No bacterial culture has been carried out using the 12 control vitreous samples.

Sample processing

Frozen aliquots of vitreous biopsies were thawed at the Rotterdam Eye Hospital and fixed in lysis buffer (NucliSens, Biomerieux, Amersfoort, the Netherlands). The mm-PCR, including the panbacterial PCR, requires 200 microliter vitreous liquid. Lower amounts can be used but with reduced sensitivity. The lysis buffer lyses bacterial and human cells and stabilizes the liberated nucleic acids. It was then sent by regular surface mail to the Regional Laboratory Kennemerland in Haarlem to be analysed by mm-PCR. DNA was isolated with magnetic beads (NucliSens; Biomerieux). Isolated DNA was mixed with PCR mastermix (LC480 probe master mix [Roche, Almere, the Netherlands]), aliquots were added to the prefilled PCR plate and the plate was loaded into the LightCycler® 480 (Roche). With every sample, a blank is isolated to establish the background signal of the PCRs.

The mm-PCR is performed in a 96-well plate on a LC480 real-time PCR instrument (Roche). The 96-well plate is prefilled with the primers and probes and stored in the freezer for use.

Mm-PCR composition

The mm-PCR composition is shown in Table 1. The composition is based on a recent series of endophthalmitis patients in settings comparable with the Dutch urban industrialized situation, as well as screens of the medical records of endophthalmitis patients at the Rotterdam Eye Hospital from 2017 to 2020 (a period later than Manning's study) (Tables 2a and 2b, results section).

Table 1. Target genes and the corresponding bacterial species targeted in mm-PCR

Target gene	Corresponding bacterial species targeted in mm-PCR
tuf	*Staphylococcus epidermidis*
tuf	*Staphylococcus aureus*
*rpo*B	*Streptococcus pneumoniae*
*aro*E	*Enterococcus faecalis*
*rpo*B	*Streptococcus mitis/oralis*
tuf	*Staphylococcus lugdunensis*
*rpo*D	*Pseudomonas aeruginosa*
cel	*Propionibacterium acnes*
*rpo*B	*Streptococcus parasanguinis*
*rpo*B	*Streptococcus sanguinis*
*rpo*B	*Streptococcus dysgalactiae*
*rpo*B	*Streptococcus salivarius*
*rpo*B	*Streptococcus mutans*
*rpo*B	*Streptococcus agalactiae*
*rpo*B	*Streptococcus salivarius*
*rpo*B	*Streptococcus pyogenes*
tuf	*Staphylococcus haemolyticus*
tuf	*Staphylococcus saprophyticus*
*fuc*K	*Haemophilus haemolyticus*
*rpo*D	*Pseudomonas putida*
*rpo*D	*Pseudomonas fluorescens*
16S rRNA	*Bacteriaceae*

Analysis of clinical samples

The mm-PCR consists of 22 single-target PCRs – twenty PCRs target each of the species enlisted in Table 1, one targets the 16S rRNA-gene that measures the total amount of bacterial DNA in the sample and one the phocid herpesvirus (PhHV) – as a process control. The *Streptococcus* species-specific PCRs target the rpoB-gene, and the *Staphylococcus* species-PCRs target the tuf-gene. The other species each are targeted by specific genes enlisted in Table 1 (Yang et al. 2002). The amplification of the species-specific PCRs is detected with RazorLight (Roche), and the amplification of the 16S rRNA gene and PhHV has a specific hydrolysis probe. The RazorLight-assisted PCRs employ a melting curve of the amplification product to identify the species.

The vitreous samples were analysed using the mm-PCR. Species specific signals of 16S rRNA positive samples were identified by Cp value and melting curve analysis. The Cp value of a positive species specific signal is expected to be approximately 2–3 Cp values higher than the 16S rRNA signal due to four to eight multiple copies of the 16S rRNA-genes.

In the series of mm-PCRs, we determined the background signal of contaminating bacterial DNA using three mock samples that did not contain patient material. These process controls were run along the whole process of DNA isolation and mm-PCR. For the series of samples enlisted in Table 3, the mean values of these three process controls in the 16S rtPCR was 32.19 with a standard deviation of 0.47. The Δ16S Cp is considered significantly higher than background when the $\Delta Cp > 4SD$, being 2 SD for the error in the determination of the blank and 2 SD for the determination of the 16S rtPCR of the individual samples. In this series of samples, the 4SD value is $4 \times 0.47 = 1.88$ Cp value, implicating that samples with a $\Delta Cp > 1.88$ are considered positive for bacterial DNA. Samples with a lower ΔCp were only considered positive when a positive signal was obtained in one of the species-specific PCRs.

When the mm-PCR was negative but significant amounts of 16S rRNA were found, the 16S rRNA V3-V4 region was amplified and sequenced to determine the bacterial species, employing BlastN on the NCBI database (Drancourt et al. 2000).

Results

Mm-PCR composition

Table 2a shows the selected studies on which the mm-PCR composition is based. We ranked bacterial pathogens according to their reported frequency. Pathogens were only included in the table if they occurred twice or more. The mm-PCR encompasses 90% of the identified species. Nine species were added that are usually not classified after culture by their species name but on their alpha- and beta-haemolytic appearance, such as the streptococci.

Mm-PCR and bacterial culture results of the clinical samples

Pathogens found in mm-PCR and in conventional culture are shown per patient in Table 3. All twelve vitreous control samples were negative in mm-PCR. Concordant results were found in 24 (89% from a total of 27) samples for culture and mm-PCR. Nonconcordant results were found in three (11%) samples. If we assume that bacterial culture is the gold standard and that the twelve control samples are culture-negative, sensitivity of mm-PCR will be 95% and specificity 94%.

Table 4 summarizes the data of the nonconcordant results in three patients with nonconcordant test results.

In the first sample (sample 1 from Table 4), the bacterial culture did not identify a pathogen; however, PCR showed the presence of *S. agalactiae,* which corresponds to the severe clinical course of the patient. Possible explanations are that this bacterial species may be fastidious in culture, there were problems with transport conditions, or too little vitreous biopsy was available for proper culture.

In the second sample (sample 2 from Table 4) mm-PCR as well as 16S rRNA were negative, with a sporadic occurrence of *S. capitis* in culture. The clinical course was compatible with both a real infection with good response to treatment and no infection. A too small aliquot may have been sent in for PCR. Loss of bacterial DNA due to long storage could be another explanation.

In the third sample (sample 3 from Table 4), PCR showed *S. pneumoniae* and culture showed *S. mitis*. Discriminating between these two pathogens is very difficult because the bacterial strains are genetically related to each other. Further analysis was performed through lytA PCR, which confirmed that the sample was positive for *S. pneumoniae* (Messmer et al. 2004).

In Table 3, the Δ*C*p value of the 16S qPCR is an indication for the amount

Table 2a. Bacterial pathogens found in the literature study with a frequency of at least 2 or more in vitreous samples of (suspected) endophthalmitis

Bacterial species found in literature study	Frequency (%) from a total of 561 samples	Included in mm-PCR
Staphylococcus epidermidis	344 (61.3%)	Yes
Staphylococcus aureus	61 (10.9%)	Yes
Streptococcus pneumoniae	32 (5.1%)	Yes
Enterococcus faecalis	21 (3.7%)	Yes
Streptococcus mitis/oralis	16 (2.8%)	Yes
Staphylococcus lugdunensis	15 (2.8%)	Yes
Haemophilus influenzae	14 (2.5%)	No
Proteus mirabilis	10 (1.8%)	No
Serratia marcescens/species	6 (1.1%)	No
Pseudomonas aeruginosa	4 (0.7%)	Yes
Abiotrophia species	3 (0.5%)	No
Propionibacterium acnes	3 (0.5%)	Yes
Streptococcus parasanguinis	3 (0.5%)	Yes
Achromobacter xylosoxidans	3 (0.5%)	No
Staphylococcus capitis	2 (0.3%)	No
Propionibacterium propionicus	2 (0.3%)	No
Gemella morbillorum	2 (0.3%)	No
Corynebacterium species	2 (0.3%)	No
Streptococcus sanguinis	2 (0.3%)	Yes
Streptococcus dysgalactiae	2 (0.3%)	yes
Streptococcus salivarius	2 (0.3%)	Yes
Proteus vulgaris	2 (0.3%)	No
Acinetobacter species	2 (0.3%)	No
Stenotrophomonas maltophilia	2 (0.3%)	No
Rhizobium radiobacter	2 (0.3%)	No
Morganella morganii	2 (0.3%)	No
Agrobacterium tumefaciens	2 (0.3%)	No
Streptococcus mutans		Yes
Streptococcus agalactiae		Yes
Streptococcus salivarius		Yes
Streptococcus pyogenes		Yes
Staphylococcus haemolyticus		Yes
Staphylococcus saprophyticus		Yes
Haemophilus haemolyticus		Yes
Pseudomonas putida		Yes
Pseudomonas fluorescens		Yes

This table summarizes data from 5 published studies and medical records form the Rotterdam Eye Hospital records at the REH from 2017 to 2020 (Chiquet et al. 2008; Pijl et al. 2010; Manning et al. 2018; de Geus et al. 2020; Kosacki et al. 2020).

Table 2b. Number of positive vitreous samples per study (from Table 2a)

Literature	Number of positive samples after culture and/or PCR
Manning et al. (2018) (NL[†])	114
Pijl et al. (2010) (NL)	166
Chiquet et al. (2008) (FR[‡])	72
Geus et al. (2020) (NL)	92
Kosacki et al. (2020) (FR)	107
REH 2017-2020 (NL)	82
Total	633*

* This discrepancy with the total amount of positive samples from Table 2a is due to the fact that in Table 1 pathogens were only included when they had a frequency of 2 or more.
[†] Dutch.
[‡] French.

of bacterial DNA in the sample. Samples with a Δ16S below 1.88 are not reliably measured positive for bacterial DNA and are indicated through underlining.

Discussion

The aim of this study was to compare mm-PCR with classical bacterial culture in the detection of pathogens in patients suspected of bacterial endophthalmitis. We demonstrated that the mm-PCR had a sensitivity of 91% and a specificity of 94% with bacterial culture as the gold standard. As bacterial culture does not have a 100% sensitivity nor specificity, we prefer to report that mm-PCR was concordant with bacterial culture in 24 of the 27 samples tested. Two out of three non-concordant samples showed positive PCR signals identifying a pathogen that corresponded to the clinical course of the patient. Though discrimination between the two pathogens in the third sample was difficult, lytA PCR confirmed that the sample containing *S. pneumoniae*.

These results demonstrate that the mm-PCR is an alternative for bacterial culture of endophthalmitis samples. Viable bacteria are needed for bacterial culture and subsequent antibiotic susceptibility testing. As mm-PCR and panbacterial PCR require only bacterial DNA to identify causative organisms, we may use mm-PCR to reverse the order of treatment of endophthalmitis, allowing intravitreal injection of empiric antibiotics at the patient's very first presentation, preferably by the referring ophthalmologist, without, importantly, losing diagnostic surveillance. Diagnostic surveillance serves to establish the diagnosis, supports the prudent use of antibiotics and allows an analysis in a cluster of infections. The preservation of the vitreous biopsy in a lysis buffer which allows sending it to the laboratory by regular mail without demands for temperature control, adds to the feasibility of this diagnostic technique.

Currently, in patients with suspected bacterial endophthalmitis, PCR techniques are being used as a diagnostic tool in addition to bacterial culture.

Several studies have reported similar or greater sensitivity rates when using PCR compared with classical bacterial culture, but often at the cost of specificity (Ugahary et al. 2004; Cornut et al. 2014).

Most of the studies published have used panbacterial 16S PCR techniques, followed by sequencing. This method, however, is still very time-consuming (2–4 days). Cornut et al. (2014) grouped the results of 16 studies and found a 40.5% identification rate for conventional culture versus 82.3% in PCR. Bacterial identification through

Table 3. Results from bacterial culture and mm-PCR per sample (n = 27)

Sample number	Bacterial culture	Mm-PCR	Δ16S	Concordancy
1	*E. faecalis*	*E. faecalis*	8.02	Positive
2	*S. epidermidis*	*S. epidermidis*	2.41	Positive
3	*S. epidermidis*	*S. epidermidis*	1.43	Positive
4	*S. epidermidis*	*S. epidermidis*	3.65	Positive
5	*S. epidermidis*	*S. epidermidis*	3.66	Positive
6	*S. epidermidis*	*S. epidermidis*	4.68	Positive
7	*S. epidermidis*	*S. epidermidis*	7.68	Positive
8	*S. epidermidis*	*S. epidermidis*	4.74	Positive
9	*S. epidermidis*	*S. epidermidis*	2.95	Positive
10	*Haemophilus influenzae*	*H. influenzae**	13.73	Positive
11	No growth	Negative	3.02	Positive
12	No growth	Negative	1.19	Positive
13	No growth	Negative	1.66	Positive
14	No growth	Negative	3.79	Positive
15	*P. aeruginosa*	*P. aeruginosa*	7.14	Positive
16	*S. aureus*	*S. aureus*	3.01	Positive
17	*S. aureus*	*S. aureus*	3.25	Positive
18	*S. aureus*	*S. aureus*	5.36	Positive
19	*S. aureus*	*S. aureus*	2.69	Positive
20	*S. dysgalactiae*	*S. dysgalactiae*	12.97	Positive
21	*S. lugdunensis*	*S. lugdunensis*	3.27	Positive
22	*S. mitis/oralis*	*S. mitis/oralis*	5.21	Positive
23	*S. mitis/oralis*	*S. mitis/oralis*	12.30	Positive
24	*S. pneumoniae*	*S. pneumoniae*	18.07	Positive
25	No growth	*S. agalactiae*	3.37	Negative
26	*S. capitis*	Negative	1.7	Negative
27	*S. mitis/oralis*	*S. pneumoniae*	10.55	Negative

* Pathogen was not included in mm-panel, determination through sequencing.

Table 4. Nonconcordant samples and their corresponding patient characteristics

Sample	Sample 1	Sample 2	Sample 3
Bacterial culture	No growth	Sporadic *S. capitis*	Sporadic *S. mitis/oralis*
Gram stain	–	A few granulocytes, no bacteria	–
Mm-PCR	*S. agalactiae*	Negative	*S. pneumoniae*
Cause of infection	Fako		
IVI	Resuturing PKPL* after blunt trauma		
Treatment	IVI antibiotics	IVI antibiotics	IVI antibiotics
Extra intervention	Vitrectomy, removal of IOL, incision encapsulated ciliary body	None	Vitrectomy and oil
Reason vitrectomy	Hypotony, to relieve traction on ciliary body	-	Funnel retinal detachment
VA pre-3/300	endophthalmitis	0.2	1.0
Presenting VA	LP+	2/300	LP+
VA post-3/300	endophthalmitis	LP+	1.0
Δ16s rRNA	3.37	1.7	10.55
Compatibility with infection	Compatible with infection	Compatible with no infection or successful treatment	Compatible with infection, but funnel RD is specific

* Perforating keratoplasty.

rtPCR on species level has not been widely investigated. Only Kosacki et al. (2020) reported the use of two specific primers for the identification on species level. In our approach, we used a mm-PCR as the main diagnostic tool with 20 primers to identify the most prevalent causative organisms of bacterial endophthalmitis on the species level.

Limitations of our study

Mm-PCR is a form of rtPCR, and rtPCR also has its limitations. When a specific PCR primer for a specific pathogen is not included in the panel, the pathogen will not be detected. We addressed this limitation by including specific primers for the most common bacterial pathogens. The set of primers used covers the detection of more than 90% of bacteria causing endophthalmitis found in selected series comparable with the Dutch urban setting. Furthermore, when no signal emerges from the mm-PCR, as a backup, panbacterial PCR followed by sequencing was performed to ensure identification of the pathogen at the species level. However, the attending ophthalmologist should be aware that primer mismatches may give a false-negative result.

H. influenzae, S. marcescens and *P. mirabilis* were also reported as frequent endophthalmitis-causing pathogens (Table 1) and should be included in the multi-mono-PCR panel in the future. Furthermore, we plan to add a panfungus primer (LSU or ITS) to the panel. The more specific PCRs are added to the panel, the less is the need for the more expensive or elaborate panbacterial sequence analysis. It is a choice of incremental gain to be made by clinicians and laboratory scientists and will depend on the pathogens prevailing in the area (Gentile et al. 2014; Joseph et al. 2019).

In our study of vitreous samples from our biobank, all vitreous samples had been taken before antibiotic treatment was started. Therefore, we could not validate that previous antibiotic treatment would not compromise our PCR analysis. Previous studies, however, have established that antibiotic pretreatment did not preclude diagnosis by PCR (Chiquet et al. 2008; Kosacki et al. 2020).

A limitation of current PCR compared with classical bacterial culture is its inability to determine sensitivity to antibiotics. However, the field of recognizing resistance genes is developing and has started to find its way into ophthalmology (Chiquet et al. 2018; Mahfouz 2020). At this moment, changing patterns of bacterial susceptibility to antibiotics will have to noticed from culture results of samples sent to the diagnostic laboratory by other

disciplines. Because most cases of endophthalmitis are not caused by nosocomial infection, high susceptibility to the current antibiotic treatment can be expected. To remain covered for all eventualities, part of the vitreous biopsy (taken after injection of antibiotics) can also be sent out for culture as resistant bacteria may still grow in culture and might help us choose an alternative antibiotic. For the individual patient, though, a change of empiric antibiotics based on sensitivity testing will generally be too late to alter the clinical course of that patient. Interestingly, a microbiological diagnosis is not primarily needed for empiric treatment as even in patients with the worst outcome in a recent series of 168 patients, the causative organisms had been sensitive to the empiric antibiotics given (Manning et al. 2018). Not the choice of antibiotics was at fault but possibly the delay in injecting them (unpublished data, van Meurs JC, Euretina, Amsterdam, 3 october 2020).

With mm-PCR, we can opt for early treatment, prevent treatment delay and gain precious hours with earlier antibiotic treatment, while maintaining diagnostic surveillance.

In conclusion, this study shows that our mm-PCR approach has a high concordance rate with the results of bacterial culture. This suggests that the mm-PCR (with panbacterial PCR as backup) is a feasible diagnostic tool in the diagnostic process of bacterial endophthalmitis. Further research with larger populations is required to validate the mm-PCR as a diagnostic tool after intravitreal administration of antibiotics.

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