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Laboratory Manager
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Dear Colleague

Changes to *Acanthamoeba* keratitis reference diagnosis service

I am writing to advise you of changes to our *Acanthamoeba* keratitis (AK) reference diagnosis service.

From April 2019, we will be offering a combined testing algorithm for the diagnosis of AK to include both *in vitro* culture / microscopy and an in-house probe-based qPCR. Validation of these methods has shown that this approach provides the greatest diagnostic sensitivity compared with either culture / microscopy or qPCR alone.

Following an extensive evaluation and validation process, we have determined that both methods are capable of detecting a single cyst in a sample. In fact, the majority of positive *Acanthamoeba* samples were positive by both methods. However, we also detected both culture positive/PCR negative and culture negative/PCR positive samples during the validation process. In addition to potential stochastic sampling effects in samples containing little material, it is likely that there are other reasons why a single diagnostic approach cannot diagnose all AK infections and there are numerous benefits to continuing to employ both methods. qPCR is a quicker diagnostic test than culture/microscopy and also allows quantitation by comparison with reference standards. PCR can also detect *Acanthamoeba* DNA in samples from treated patients which may not be amenable to culture. On the other hand, culture/microscopy can provide a species designation based on cyst morphology for positive samples whereas PCR provides identification to genus level only. Perhaps more importantly, culture/microscopy can detect and identify species not amplified or detected by the current PCR primers and probe.

For positive samples, an archive of *Acanthamoeba* culture material and DNA will be retained, wherever possible, for future genotyping studies and potentially also for drug resistance investigations.

We are recommending the combined algorithm however culture/microscopy will still be offered alone for primary samples and for confirmation / species identification on positive culture specimens *if specifically requested by the sending laboratory*. PCR will NOT be offered alone. Please note: from April 1st 2019, we will no longer be able to supply Page's agar culture plates.

Please see the attached guidelines for referral of specimens for *Acanthamoeba* investigation. All samples should be accompanied by a specific *Acanthamoeba* DPL referral form, a copy of which is also enclosed, and which will be available to download and print from: www.parasite-referencelab.co.uk from 1st April 2019.

From 1st April 2019, charges for laboratory investigation of *Acanthamoeba* keratitis will be as follows:

- For combined culture + PCR £75
- For culture only £30

Charges for private testing can be obtained on request.

The turnaround time for the combined testing will be 7 working days: samples for AK qPCR will be batched and run weekly. Cultures will be set up daily, as per current practice. Positive results from either qPCR or culture/microscopy will be telephoned in advance of the final combined result.

Please do not hesitate to contact me if you require any further information.

Yours sincerely

Claire Rogers