

Comment on: Sensitivity and specificity of potassium hydroxide and calcofluor white stain to differentiate between fungal and *Pythium* filaments in corneal scrapings from patients of *Pythium* keratitis

Dear Editor,

We read the interesting article by Bagga *et al.*^[1] on sensitivity and specificity of potassium hydroxide (KOH) and calcofluor white (CFW) stain for differentiating fungal and *Pythium* filaments in corneal scrapings from patients of *Pythium* keratitis. We have a few observations and suggestions to make it an interesting read for the benefit of all ophthalmologists.

Firstly, the authors have mentioned that the images of KOH + CFW were retrieved from the microbiological database distributed randomly and evaluated by three microbiologists. It will be interesting to know whether it was a retrospective study design or prospective or retrospective data analyzed prospectively.

Secondly, the authors have taken culture as the gold standard for assessing the sensitivity and specificity of two stains. It would be important and relevant to know if the same microbiologist amongst the three evaluated culture results to understand the possibility of subjective bias in the study.

Thirdly, we believe that in the methodology it would have been useful if the authors could have characterized the differentiating features of *Pythium* and fungus on smearing. The *Pythium* filaments on 10% KOH wet mount appear long, slender, hyaline, aseptate, or sparsely septate with perpendicular lateral branches. In contrast, fungal filaments appear broad, sparsely septate with branching at various angles. Numerous vesicles within the hyphae can be observed in both. Hence, the branching of hyphae is an important differentiating characteristic for the two species.^[2] Moreover, the thin-walled appearance collapsed, and ribbon configuration is perhaps more evident on the KOH + CFW combination, which is a definite add-on to the microbiological analysis.^[3] Additionally, iodine-potassium iodide (IKI)-sulfuric acid stain (IKI-H₂SO₄) can differentiate *Pythium* filaments that appear blue from fungal filaments, which appear yellow or brown.^[4]

Fourthly, although equally experienced (5 years), the sensitivity of three microbiologists differed by a good margin (96.55% vs. 89.66% vs. 79.31%). What could be the potential reason for this? Whether it was the bias toward gold standard culture, contrast, quality of microbiological smears, or interobserver difference?

Lastly, [Fig. 2] shows the comparison between fungal (b and d), *Pythium* (c and d) filaments in KOH (a and b), and KOH + CFW (c and d) mounts. It should be fungal (b and c) probably, there is a mismatch.

Acknowledgments

Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Pondicherry.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Access this article online	
Quick Response Code:	Website: www.ijo.in
	DOI: 10.4103/ijo.IJO_345_22

Cite this article as: Gurnani B, Kaur K. Comment on: Sensitivity and specificity of potassium hydroxide and calcofluor white stain to differentiate between fungal and *Pythium* filaments in corneal scrapings from patients of *Pythium* keratitis. *Indian J Ophthalmol* 2022;70:2204.

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