

Comparison between Lactophenol Cotton Blue and Iodine Glycerol for Identification of Fungal Elements in Clinical Samples

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Abstract

Fungal infection is alarming phenomenon now a day because of increasing number of patients. Lactophenol Cotton Blue is widely used for staining, but there is a necessity to develop an alternate equipotent stain which can replace it due to its high level of tumorigenic and hazardous nature. Iodine glycerol is better alternative for it. Iodine glycerol is safe, eco-friendly, good visual clarity, with better staining properties. Hence, we got here various clinical samples which were analysed simultaneously using Lacto-phenol Cotton Blue and Iodine Glycerol by using different techniques like teasing technique, slide culture technique and adhesive tape technique. Parameters like degree of transparency, visual clarity, resolution, contrast, staining characteristics like uniformity, formation of artefacts were analysed for better demonstration of fungal morphology. Iodine-Glycerol is a better alternative to Lacto-phenol Cotton Blue for the demonstration of fungal morphology in the clinical microbiological laboratories. It is eco-friendly, noncarcinogenic and much potent staining reagent. It is necessary to carry further research as there are no specific guidelines regarding the preparation of the Iodine-Glycerol staining reagent.

Keywords: LPCB, Iodine Glycerol; Lugol's Iodine; Fungal Morphology; Transparency; Staining Characters.

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Introduction

For identifying filamentous fungi based on their characteristic morphological features, Microscopic observation of wet mounts is the most widely used method in clinical microbiology laboratories. Most common technique used is lactophenol cotton blue tease method. Lactophenol cotton blue is preferred universally for its usage as a fixative, staining and mounting methods. The phenol component of lactophenol cotton blue is carcinogenic and hence it is imperative to search an alternate staining reagent. Hence, need arise for alternative safe and equally functioning fungal mount method.

Lugol's Iodine is a potent fungicide as it reacts with thiol groups of fungal enzymes and proteins and hence can functionally replace the phenol component in Lactophenol cotton blue. Although iodine solution in combination with chloral hydrate (Melzer's solution) is currently being used in clinical microbiological laboratories, it has never been used as a mounting method for microscopic identification of fungi (Baszowski et al., 2006). Also chloral hydrate is known to be a hazardous substance. The Iodine component in Lugol's Iodine stains the outer wall of fungus and can functionally replace cotton blue as a staining reagent.

Addition of 0.25% pure Glycerol to Lugol's

Iodine can potentiate the hygroscopic nature of the Glycerol-Iodine. Hence, we examined the possibility of using iodine-glycerol as an alternative to LPCB and to evaluate its usefulness for wet mount preparations for microscopic observation and identification of certain clinical isolates of filamentous fungi.

Materials and Methods

The present study was conducted from May 2017 to October 2017. Institutional Ethical committee approval was taken before conducting the study. Lactophenol Cotton Blue, Lugol's Iodine and pure Glycerol were purchased from Hi-Media. 0.25 ml of pure Glycerol was added to 99.75 ml of distilled water to prepare 0.25% Glycerol. Equal quantities of 0.25% Glycerol and Lugol's Iodine were added to prepare the final stain Glycerol-Iodine. 64 clinical samples were processed. corneal scrapings, bits of tissue, nail clippings, hair plucks, sputum, bronchial washings, skin scrapings, etc were samples for fungal infection. skin scrapings, corneal scrapings, sputum etc were kept in 10% potassium hydroxide for 30 minutes to dissolve the cementing substance holding the keratinised cells followed by thorough analysis under low power field for the presence of fungal elements. nail clippings, bits of tissue etc were kept in 40% potassium hydroxide and left incubated overnight at 37°C followed by analysis for fungal elements. nail clippings, bits of tissue etc were kept in 40% potassium hydroxide and kept in incubator overnight at 37°C followed by analysis for fungal elements. along with this lactophenol cotton blue and glycerol-iodine mounting were done, Various criteria like the degree of transparency, staining characteristics, visual clarity

Table 2:

	sensitivity	specificity	Positive predictive value	Negative predictive value
Glycerol iodine wet mount	60.5%	97.8%	91.2%	92.6%
Lactophenol cotton blue	55.7%	95.1%	82.2%	88.8%



Fig. 1: Lactophenol Cotton Blue and Iodine-Glycerol stains

and better demonstration of the morphology of the fungus under study are taken as measuring points to compare the efficacy of Iodine Glycerol with Lactophenol Cotton Blue stain.

Results and Discussion

The visual clarity and the degree of transparency were more with Iodine-Glycerol as compared to Lactophenol Cotton Blue. Contrast is reasonably good with Lactophenol cotton Blue. resolution is observed same with both. The staining characteristics like uniformity, clarity of the various morphological structures, lack of any artefacts due to the staining material on prolonged storage etc were better appreciated with Iodine - Glycerol than lactophenol cotton blue.

The observations in my study correlated with that of Vignesh et al., (2008) and Vacharavel, shamly et al., (2014).

We could not get adequate references due to dearth of information on the topic.

Table 1:

Number	Parameter	Iodine-Glycerol	Lactophenol Cotton Blue
1	contrast	poor	better
2	staining	good	poor
3	Uniform staining	excellent	good
4	resolution	good	good
5	Degree of transparency	more	less
6	Visual clarity	better	good
7	Demonstration of Morphology	better	good
8	Artefacts	less	more

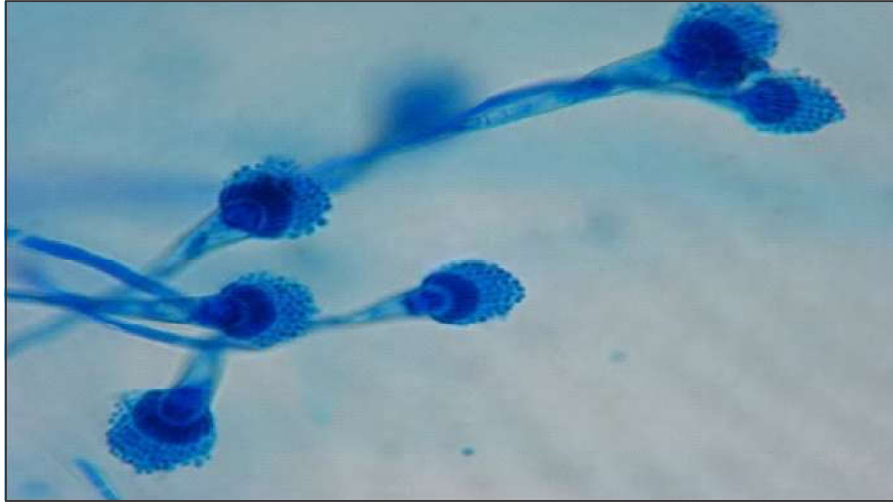


Fig. 2: LPCB of aspergillus flavus



Fig. 3: Iodine-Glycerol mount of Microsporium gypseum

Conclusion

Iodine-glycerol preparation was found to be a better technique for identification fungal isolates which may be employed as a non-hazardous and safer alternative to LPCB for fungal identification.

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