INDIGENOUS FLUORESCENT STAIN FOR FUNGUS IN SPECIMENS

Dr.K.R.L.SuryaKirani ,Prof.&HOD,Microbiology, Dr.V.Satya Chandrika,PG in Microbiology

Rangaraya Medical College,Kakinada

Abstract:

Introduction:Mycotic infections are gaining importance in the present day medicine and definite demonstration of fungus is essential for diagnosis.Small numbers of organisms in smear are identified by fluorescence microscopy.Calcofluor white fluorescent stain, a textile brightener mixed with Evans blue, is expensive and not easily available.**Aim**:To assess the sensitivity,specificity,NPV,PPV of Indigenous stain for fungus inrelation to conventional fluorescent stain,histopathology and culture.**Materials and methods**:100 cases of suspected dermatophytosis and 15 cases of systemic mycosis were included in the study.The local whitener,Ranipal with added Robin blue, another brightener was used to stain teased fungal cultures.Skin, hair and nails require pre-treatment with KOH.Biopsy slides require de-paraffinisation & pre-treatment with KOH before staining.Conventional Calcofluor stain ,histopathology and culture was done .**Results:**The results are consistantly comparable with conventional stain.Sensitivity100%,specificity93%,NPV85%,PPV100% compared to culture as gold standard. Results are comparable with Histopathology.Cost effective compared to commercial stains.**Conclusion:**Indigenous stain can be used for screening of fungus in dierect samples,biopsies as alternative in resource poor laboratories.

Key-words: Calcoflour, fluorescent, fungal stain.

Key Messages: Cost effective, easily available, efficient fluorescent stain for fungus in all types of Specimens including biopsy material.

Introduction:

Mycotic infections are gaining importance in the present day medicine due to the increasing incidence of Immunocompromised patients. Definite demonstration of fungus is important, as anti fungal treatment is expensive, long term, and associated with side effects.Sometimes it is difficult to identity the causative agents when they are present in small numbers in the sample by conventional diagnostic methods like KOH wet mount, LCB, India ink preparation for CSF .

Calcofluor white fluorescent stain is routinely used for demonstration of fungus. It is a textile brightener mixed with Evans blue. Calcofluor white (CFW) selectively binds to the cellulose and chitin of the fungal cell wall and when visualized under fluorescent microscope gives apple green fluorescence.

Materials and Methods:

Calcofluor white is expensive and not easily available. There are studies abroad with different other local brighteners.So, I have tried the local whitener, to stain teased fungal cultures. It was good but there was non-specific fluorescence. So, I added another brightener.

COMPOSITION:

Conventional calcofluor stain

Stock Solution A :

calcofluor stain 1.9gms

Distilled Water 100ml

Stock Solution B:

Evans blue 0.05gms

Distilled Water 100ml

Working Solution :

Solution A 1ml

Solution B 9ml

Indigenous Fluorescent stain

Stock Solution A :

RANIPAL 1.9gms

Distilled Water 100ml

Stock Solution B:

ROBIN BLUE 0.05gms

Distilled Water 100ml

Procedure

1. One drop of working solution placed on a clean glass slide.
2. Small amount of specimen taken and emulsified.
3. Cover slip is placed to obtain as thin a film as possible.
4. Viewed under fluorescent microscope after 5 mins..

Skin, hair and nails require pre-treatment with KOH.

- Slides immersed overnight in 10% KOH solution. Working solution added to slide & allowed to act for 10 minutes. Excess stain drained, slide washed. Drop of fresh working solution added.
- Coverslip placed over smear.

Biopsy samples require de-paraffinisation& pre-treatment with KOH before staining.

100 cases of clinically suspected dermatophytosis cases were studied by conventional stain,indigenous stain and culture.

15 de-paraffinized tissue section smears from clinically diagnosed fungal infections were submitted to fluorescent staining with the indigenous stain and compared with histopathology.

Results:

This stain gives Apple green /blue green Fluorescence to the fungi.

Stain also produces a contrasting dark background, there by enhancing the detection of fungi.

This stain has given wonderful results with fungal cultures, fungal elements in samples.

Table I:Comparison with conventional stain

n=100

Stain	Positive	Negative
Conventional	35	65
Indigenous	35	65

Results are consistant in all the cases with conventional stain.

Table II: Comparision with culture

n =100

Method	Positive	Negative
Culture	30	70
Indigenous stain	35	65

Sensitivity = 100%
Specificity = 93.3%
Negative Predictive Value=85%
Positive Predictive Value =100%

TableIII:Comprison with Histopathology

n=15

Method	Positive	Negative
Histopathology	10	5
Indigenous stain	12	3

Results are comparable with Histopathology

Table IV-Comparison of Indigenous stain and histopathology section

Sl. No.	Source of tissue section	Fungus observed with Fluorescent Stain	Fungus observed with Histopathological Stain
1	Biopsy - Sinus	*Mucor*	*Mucor*
2	Biopsy – Polyp nose	*Rhinosporidiumseeberi*	*Rhinosporidiumseeberi*
3	Biopsy – Sinus	*Aspergillus*	Doubtful
4	Biopsy – Sinus	Negative	Negative
5	Biopsy – Bronchus	*Aspergillus*	*Aspergillus*
6	Biopsy – Cervix	*Candida*	*Candida*
7	Biopsy – Foot	Negative	Negative
8	Biopsy – Bronchus	*Candida*	*Candida*
9	Biopsy – Sinus	*Mucor*	*Mucor*
10	Broncho alveolar lavage fluid	*Pneumocystis carinii*	Negative
11	Biopsy – Nasal mass	*Aspergillus*	*Aspergillus*
12	Biopsy – Sinus	Negative	Negative
13	Biopsy – Sinus	*Mucor*	*Mucor*
14	Biopsy – Cervix	*Aspergillus*	*Aspergillus*
15	Biopsy - Sinus	*Aspergillus*	*Aspergillus*

- Out of 15 de-paraffinised tissue sections stained with the indigenous stain, 12 showed fungi.
- Out of 12 positive smears for fungus, 5 were *Aspergillus*, 3 were *Mucor*, 2 were *Candida*, and 1 each were *Rhinosporidiumseeberi* and *Pneumocystis carinii*.
- Out of 12 positive tissue sections by fluorescent microscopy, only 10 were positive by standard histopathological staining technique.
- One doubtful and one negative tissue section by standard histopathological staining technique were shown to be positive for fungus by the indigenous fluorescent stain.

Discussion:

Fluorescent microscopy:Organisms easily focused in screening large number of samples.Less eye strain.More magnification with dry objectives,so larger area of the slide is screened.Small numbers of organisms in smear are also identified---sensitive.

INDIGENOUS STAIN:

Time required for staining as well as screening for fungus is less when compared to histopathological staining. More economical than histopathological staining when fluorescent microscope is available.

Components used in conventional CFW stain are not readily available,Veryexpensive,Carcinogenic – extreme care must be taken.

Indigenous stain:Readily available ,Very cheap,Not harmful to handle.

Trained techinician can identify the presence of fungus.Expert is required for identification of fungus.

It helps in rapid diagnosis,more so if it is in-situ-identification. Most useful for *Fungi in specimens and cultures, in CSF,Blood cultures,*

Table V:Comparison of cost of traditional cfw stain & indigenous stain

Classic CFW Stain		Indigenous Stain	
Company Name	Price (INR)	Company Name	Price (INR)
Sigma F3543-1G Fluorescent Brightener 28 Synonym: Calcofluor White M2R, Tinopal UNPA-GX	4125.00 per 1 g	Ranipal Fabric Brightener	12.00 per 25 g
Sigma 46160-5G-F Evans Blue	2450.00 per 5 g	Robin Blue	32.00 per 100g
Total Cost to make 10 ml of working solution	80.58/-	Total Cost to make 10 ml of working solution	0.154/-

Readymade stain(Sigma) --100ml –Rs.1700/-.

10ml—Rs.170/-

Cost per 10ml solution of Indigenous stain is negligible as compared to commercial stain.

Stains should be fresh,Sample should be fresh. Should be seen immediately.The reduction of emission intensity is called ‘fading’. It could be because of either ‘photobleaching’ or ‘quenching’.

To prepare and throw away the stain everyday becomes evenmore expensive with conventional stain and very negligible with indigenous stain.

The previous workers who have used local whiteners found background non specific fluorescence.In Indigenous stain this noise was removed by adding another local brightener.

CONCLUSION

This indigenous stain can be applied to any sample where the demonstration of fungus is desired.In a developing country like India, the prohibitively high cost of conventional fluorescent stains like CFW prevents the use of fluorescent microscopy in most institutes, despite the availability of fluorescent microscopes and expert personnel. The present indigenous stain overcomes this hurdle and definitely very useful, given its low cost and higher sensitivity when compared to routine stains.This stain has given wonderful results with fungal cultures, fungal elements in samples with small work up like that Skin, hair and nails require pre-treatment with KOH .Biopsy samples require De –paraffinisation& pre-treatment with KOH before staining.

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