Evaluation of boric acid as a mounting agent in a microbiology laboratory

Shweta Singh, Sayan Bhattacharyya, Asim Sarfraz, Dhirendra Kumar, Nahid Anjum, Ankur Kumar

From Department of Microbiology, All India Institute of Medical Sciences, Patna, Phulwari Sharif, Patna, Bihar, India

Correspondence to: Dr. Sayan Bhattacharyya, Department of Microbiology, All India Institute of Medical Sciences, Patna, Phulwari Sharif, Patna, Bihar, India. Phone: +91-9006621729. E-mail: sayantheboss@yahoo.co.in

Received – 11 June 2017 Initial Review – 16 June 2017 Published Online – 09 July 2017

ABSTRACT

Introduction: Boric acid is a low-toxicity, non-volatile mineral with is antiseptic, antifungal, and antiviral properties. It is used as a wettable powder, liquid (applied as a spray or aerosol), emulsifiable concentrate, granules, powders, dusts, pellets, tablets, paste, bait or crystalline rods, depending upon the circumstances and target. In light of this information, the present study is aimed to compare bacterial and fungal mounts with both lactophenol cotton blue (LCB) and Boric acid-methylene blue-glycerol (BMG) i.e., effectivity of boric acid as mounting agent since it is a disinfectant, antiseptic, preservative and suppresses conidiogenesis in fungi. **Material and methods:**This mounting agent of boric acid was compared with lactophenol cotton blue commercial solution. Results: BMG was found to be equally effective in comparison with LCB. **Discussion:** BMG can be a cheaper alternative to LCB for observing colonies from Saboraud's dextrose agar.

Key words: *Boric acid, Mounting agent, Lactophenol cotton blue*

The conventional laboratory techniques for the laboratory investigation of mycotic infections involve the use of lactophenol cotton blue stain (LCB) as mouting agent. The LCB mounts are prepared to examine detailed microscopic morphological features of fungi grown on culture media [1]. Some other stains can also be used for this purpose. Boric acid (BA) is a low-toxicity, non-volatile mineral with appreciable antiseptic, antifungal, and antiviral properties. It is used as a wettable powder, liquid (applied as a spray or aerosol), emulsifiable concentrate, granules, powders, dusts, pellets, tablets, paste, bait or crystalline rods, depending on the circumstances and target [2]. BA is a naturally occurring, white powder found naturally in deserts of California (USA) and other places. It is a mild acid. BA, as 2% aqueous solution, is a safe and fairly effective agent that may be used on mucous membranes (1 tablespoon/quart of water) [3]. It is also useful as an eye wash, vaginal suppositories, or as a douche for chronic yeast infections, used topically as a skin protection [3,4]. It is insecticidal, also an excellent fire retardant (used in cellulose insulation, etc.,) and wood preservative [3-5].

It has been considered as a safer alternative material to highly volatile, synthetic chemical pesticides [2]. It has some disadvantages by the fact that has been linked to adverse health effects such as respiratory irritation that can occur from chronic inhalation of airborne BA or borates. Researchers showed eye irritation, dryness of the mouth, nose, or throat, sore throat, and cough at mean exposures of 4.1 mg/m [3,2]. In light of this information, the present study is aimed to compare bacterial and fungal mounts with both LCB and Boric acid-methylene blue-glycerol (BMG), i.e., the effectivity of BA as mounting agent since it is a disinfectant, antiseptic, preservative, and suppresses conidiogenesis in fungi [6]. LCB is the conventional mounting agent for studying fungal colonies from sabouraud dextrose agar slant but is quite costlier as compared to BA powder [7,8].

Aim

This study aims to evaluate BMG as mounting agent for fungal identification from samples.

Objectives

To see and compare bacterial and fungal mounts with both LCB and BMG.

MATERIALS AND METHODS: (INCLUDING TIME, PLACE AND TYPE OF STUDY)

This was a laboratory-based observational study, conducted during from October 2016 to May 2017 in the departmental laboratory.

For the purpose of mounting the following preparations of BA was used.

- 0.5 g of BA + 48 ml autoclaved deionized water + 1 ml glycerol + 1 ml methylene blue solution (0.4%)
- 0.5 g BA + 46 ml autoclaved deionized water + 2 ml glycerol + 2 ml methylene blue solution (0.4%).

This mounting agent was compared with LCB commercial solution (HiMedia, India) visually. 25 clinical, isolates each of *Pseudomonas aeruginosa, Candida albicans, Aspergillus flavus,*

and *Staphylococcus aureus*, identified by standard methods were examined by both mounting methods. Mounted slides were also kept uncovered in the laboratory at room temperature for 2 days and then viewed again to check for drying and hygroscopic properties of mounts. All tests were done at least thrice to rule out bias.

RESULTS

Boric acid-based mounting solution was equally effective as compared to LCB. In many instances, the micromorphology was better observed by BMG as compared to LCB. Results have been enumerated in Table 1.

Both the concentrations of BA were comparable to that of LCB commercial solution for all the 20 clinical isolates each of *P. aeruginosa, C. albicans, A. flavus,* and *S. aureus.* Mounted slides were also kept uncovered in the laboratory at room temperature for 2 days and then viewed again to check for drying and hygroscopic properties of mounts. The hygroscopic properties of both the mounts were comparable.

Table 1: Findings of the BMG mount and comparison with LCB

Date	*P. aeruginosa*		*C. albicans*		*A. flavus*		*Staphylococcus aureus*	
	BMG	LCB	BMG	LCB	BMG	LCB	BMG	LCB
04/10/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
18/10/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
25/10/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
01/11/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
10/11/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
16/11/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
22/11/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
03/12/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
08/12/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
15/12/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
22/12/16	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining

(Contd...)

Table 1: (*Continued*)

Date	*P. aeruginosa*		*C. albicans*		*A. flavus*		*Staphylococcus aureus*	
	BMG	LCB	BMG	LCB	BMG	LCB	BMG	LCB
07/01/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
11/01/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
19/01/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
28/01/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
04/02/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
14/02/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
22/02/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
01/03/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
04/03/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
16/03/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
23/03/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
12/03/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
19/03/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining
02/05/17	Bacilli, uniformly staining	Bacilli, uniformly staining	Budding yeasts, uniformly staining	Budding yeasts, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Septate hyphae with vesicle and conidia, uniformly staining	Cocci in clusters, uniformly staining	Cocci in clusters, uniformly staining

BMG: Boric acid-methylene blue-glycerol, LCB: Lactophenol cotton blue, *P. aeruginosa: Pseudomonas aeruginosa, C. albicans: Candida albicans, A. flavus: Aspergillus flavus, S. aureus: Staphylococcus aureus*

DISCUSSION

BA, besides being used as household disinfectant, has also been used as antibacterial agent to kill bacteria infecting fish [9]. This chemical, BA and its salts, borates, have been used in medicine as bactericidal agents, fungicides, and antiseptics since way back in the 1860s [3]. Other uses of BA are as a transport medium, as bacteriostatic agent for transporting urine samples [10]. There was no evidence that borate is toxic to the urinary pathogens encountered in naturally infected urine. Borate also preserved white blood cell morphology in urine, as found in that study, and therefore, marginally improved the diagnosis of pyuria [10]. However, the aspect of boric as mountihng solution in medical microbiology laboratory has till now not been evaluated. So our study addressed these things. Thus, boric acvid can safely be used as a cheaper alternative to LCB for observing micromorphology of bacteria and fungi from colonies on Sabourauds' dextrose agar. Further studies, however, are warranted in this area, which is very interesting and applicable in laboratory, especially in developing countries.

REFERENCES

1. Chander J. Textbook of Medical Mycology. 3rd ed. New Delhi: Mehta Publisher; 2012. p. 523.
2. EPA. Boric Acid and Its Sodium Salts. Reregistration Eligibility Document. Office of Pesticide Programs. Washington, D.C, U.S: EPA; 1993.
3. Quarles W. Boric acid, borates and household pests. IPM Pract. 2001;23:1-12.
4. Quarles W. Borates Provide Least-Toxic Wood Protection: The IPM Practioner. Vol. 14. Berkeley, CA: Bio-Integral Research Center; 1992. p. 1-11.
5. Ministry of Health R Republic of Trinidad and Tobago: Manual Infection Prevention and Control Policies and Guidelines for Health Care Services; 2011.
6. See AS, Salleh AB, Bakar FA, Yusof NA. Risk and health effect of boric acid. Am J Appl Sci. 2010;7:620-7.
7. Lacto Phenol Blue Solution. Available from: http://www.sigmaaldrich.com/catalog/product. [Last accessed on 2017 Jun 11]
8. Pioneer Boric Powder, 500 Grams. Available from: http://www.amazon.in/Pioneer-Boric-Powder-500-gm/dp/B01BIVNDM6. [Last accessed on 2017 Jun 11]
9. Ali SE, Thoen E, Evensen Ø, Skaar I. Boric acid inhibits germination and colonization of *Saprolegnia spores in vitro* and *in vivo*. PLoS One. 2014;9(4):e91878.
10. Lum KT, Meers PD. Boric acid converts urine into an effective bacteriostatic transport medium. J Infect. 1989;18(1):51-8.

Funding: None; Conflict of Interest: None Stated.

How to cite this article: Singh S, Bhattacharyya S, Sarfraz A, Kumar D, Anjum N, Kumar A. Evaluation of boric acid as a mounting agent in a microbiology laboratory. East J Med Sci. 2017;2(2):29-32.