

## BORIC ACID AS A SELECTIVE BACTERIO- STATIC AGENT

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IN 1921 Levine reported on the influence of boric acid on the growth of *B. coli* and *B. lactis aerogenes*. He found that in a fluid medium of 1 per cent. peptone water and 0.63 per cent. boric acid *B. coli* multiplied slowly while *B. lactis aerogenes* died off. He states, however, that in his subsequent studies he found that the difference in concentration of boric acid, which had an inhibitory effect on *B. lactis aerogenes*, and which did not inhibit *B. coli*, was so close that it could not safely be employed as a selective agent.

Later (1934), in his work with Epstein & Vaughn, he suggested the use of a boric acid medium which showed differentiation in that 97.3 per cent. of his *B. coli* strains grew with the production of gas, while only 6 per cent. of his strains of the genus *Aerobacter* produced gas in 48 hours at 37°C., and gas was not produced by any of the fifty-seven strains tested of the genus *Citrobacter*.

Levine's experience as regards the selectivity of boric acid when used alone is in accordance with the facts observed by me. I have found when boric acid, in a concentration of 0.5 per cent., is added to peptone water the growth of *B. coli* and of *B. lactis aerogenes* is less profuse, or is suppressed. If, however, a fermentable substance is present, e.g. lactose, while the suppression of many strains of *B. lactis aerogenes* is still maintained, the growth of most strains of *B. coli* is profuse. A similar observation with regard to the use of sodium sulphite has been reported on by Wilson (1933).

Working with boric acid, in the presence of a fermentable substance, I have found that while it shows a marked degree of selectivity, suppressing most strains of *B. lactis aerogenes* while allowing a profuse growth of most *B. coli* strains, yet it is not unusual to find a strain of *B. coli* which is susceptible, or sensitive, to the action of boric acid in a concentration of 0.5 per cent., or a strain of *B. lactis aerogenes* which can tolerate boric to this extent. A means of overcoming this difficulty can, however, be found when boric acid is used in conjunction with sodium sulphite. Table I illustrates this point. It shows, on a representative collection of the cultures used for this work, the effect of boric acid when used alone, and when used in conjunction with sodium sulphite.

Thus by the addition of sodium sulphite to a lactose boric-acid peptone water, it is possible by a suitable combination of these two chemicals to overcome to a large extent the overlapping which occurred in the results when

boric acid was used alone; and by this means to make possible a more definite line of demarcation between these two organisms.

In a medium consisting of 100 c.c. of 1 per cent. peptone water, 0.25 g. lactose, 0.5 g. boric acid and 1 g. sodium sulphite (anhydrous), the growth of *B. coli* is but little hampered, while that of *B. lactis aerogenes* is inhibited.

The medium has been tested on some 494 strains of lactose-fermenting organisms: 450 strains isolated from specimens of water, thirty-six from separate samples of human faeces, five from the excreta of cattle, one from a milk supply, one from a specimen of urine and one from a specimen of vomit. The technique employed for the isolation of the organisms was such as would

Table I

	Koser's medium		Boric alone	Boric sulphite	Indol	M.R.-V.P.	
	24 hrs.	48 hrs.					
<i>Escherichia B. coli</i>	-	-	+	+	+	+	-
G.E. faecal strain	-	-	+	+	+	+	-
Burrows' faecal strain	-	-	-	+	+	+	Sensitive to boric alone
Sloan faecal strain	-	-	+	+	+	+	-
Boylan from urine	-	-	+	+	+	+	-
Excreta of heifer No. 1	-	-	-	+	+	+	Sensitive to boric alone
Excreta of heifer No. 2	-	-	+	+	+	+	-
Excreta of heifer No. 3	-	-	+	+	+	+	-
Excreta of cow No. 4	-	-	-	+	+	+	Sensitive to boric alone
Excreta of cow No. 6	-	-	+	+	+	+	-
Armagh 2 A faecal strain	+	+	+	-	-	+	Tolerant of boric alone
Armagh 4 A faecal strain	+	+	+	-	-	+	Tolerant of boric alone
Armagh 5 A faecal strain	-	+	-	-	-	+	-
Armagh 11 A faecal strain	+	+	-	-	+	+	-
Armagh 14 A faecal strain	+	+	-	-	+	+	-
<i>B. lactis aerogenes</i>	+	+	-	-	+	+	-
Morrison faecal strain	+	+	-	-	-	+	-
Dognes water strain	+	+	-	-	-	+	-
Glenade water strain	+	+	-	-	-	+	-
Hole in well	-	+	-	-	-	+	-
Marle water strain	+	+	+	-	-	+	Tolerant of boric alone
McAdam water strain	+	+	-	-	+	+	-
Cranfield water strain	-	+	-	-	-	+	-
Vomit strain	-	+	-	-	+	+	-
Agnes	+	+	+	-	-	+	Tolerant of boric alone
No. 365 from milk	+	+	-	-	+	+	-

be feasible in any laboratory called upon to deal with a large number of specimens. In the isolation of the organisms from water, the procedure recommended by Wilson (1933) was followed; 20 c.c. of each sample was planted into "Medium A". The composition of this medium is: water 1000 c.c., peptone 10 g., lactose 10 g., sodium citrate 10 g., sodium taurocholate 10 g., Bacto agar 20 g. and 10 c.c. of 1 per cent. aqueous solution of neutral red. A representative number of the colonies developing was picked off on to bile salt-lactose agar plates, and then subcultured on agar slopes. In dealing with the other specimens, they were planted directly on the surface of bile salt-lactose agar plates and then subcultured on agar slopes. In examining these cultures, tubes containing about 20 c.c. of the lactose boric sulphite peptone water received an inoculum of the organism as heavy as was possible, yet guarding

against any clouding of the medium. At the same time tubes of Koser's (1923) synthetic citrate medium, Difco M.R. V.-P. and Difco tryptophane broth (dehydrated), were seeded; the results are recorded in Table II.

Table II

	Total No. examined	B.S.		V.-P.		B.S.		V.-P.		B.S.		V.-P.	
		+	-	+	-	+	-	+	-	+	-	+	-
Comparing the results of the boric sulphite tubes with those of the V.-P.	494	+	-	+	-	+	-	+	-	+	-	+	-
		220		12		156		106					
Comparing boric sulphite with indol results	494	B.S.	I.	B.S.	I.	B.S.	I.	B.S.	I.	B.S.	I.	B.S.	I.
		+	+	+	-	-	-	-	-	-	-	-	+
		219		14		208		53					
Comparing boric sulphite with Koser's medium	494	B.S.	K.	B.S.	K.	B.S.	K.	B.S.	K.	B.S.	K.	B.S.	K.
		+	-	+	+	-	+	-	+	-	+	-	-
		201		31		196		66					

The Voges-Proskauer test was carried out with Difco bacto M.R. V.-P. medium (dehydrated) and 40 per cent. KOH. Had it been possible to use Barritt's (1936) intensification of the Voges-Proskauer test by the addition of  $\alpha$ -naphthol, it is most likely that in the comparison of the boric sulphite and the Voges-Proskauer test, the number of boric sulphite negative and Voges-Proskauer negative results would have been greatly reduced and a large number of these organisms would have been assigned to the boric sulphite negative Voges-Proskauer positive group.

In the comparison between the boric sulphite results and the results of the indol test, there is an interesting positive and negative correlation.

SUMMARY

A fluid medium is described, containing in every 100 c.c. of peptone water 0.25 g. lactose, 0.5 g. boric acid and 1 g. sodium sulphite (anhydrous). It shows a marked selective action.

The test gives satisfactory readings within 24 hours of incubating at 37°C. I am of the opinion that all strains capable of growth in this medium may be regarded as of definite sanitary significance; they are most likely of faecal origin, while those which fail to grow are of minor sanitary importance.

I hope that the boric sulphite test may prove a useful adjunct to the bacteriological examination of samples of water.

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