entire unit can be easily dismantled, folded up and stored in a drawer when not in use. 2) The hood can be moved from laboratory to laboratory by one person. 3) It is easily decontaminated with formaldehyde vapors or many types of liquid disinfectants. If a large size gas sterilization chamber is available the entire unit can be placed in it for sterilization. 4) The cost is low. The actual cost of the materials (labor not included) is approximately $10.00.

SUMMARY

An inexpensive, collapsible plastic safety hood has been designed and constructed for enclosing certain hazardous operations with infectious microorganisms. It can also be used for operations with certain dangerous or toxic chemicals. Ventilation can be provided by attaching suitable inlet and outlet filters and the hood may be operated at a slight reduced pressure to prevent the outward escape of air-borne particles. An air lock is provided for the entrance and exit of materials. The design shown is for use by one technician who works through attached rubber gloves. Larger designs to accommodate several workers and different shapes to conform to certain enclosed equipment are possible. Fabrication of the units can be done cheaply by most fabricators of plastic products for around $25.00 per unit. The authors will supply further information and design drawings on request.

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A Selenite Brilliant Green Medium for the Isolation of *Salmonella*

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Selenite F broth is widely used as an enrichment medium for the isolation of *Salmonella*. Although the broth supports good growth of these organisms and inhibits the development of a variety of accompanying bacteria, it has a number of deficiencies (Leifson, 1936). Important among these are the lack of inhibition of *Proteus* strains and the delay of growth of *Escherichia* strains for only a few hours. Both of these groups of bacteria are commonly found in association with *Salmonella* and are a major source of difficulty in the isolation and identification of *Salmonella*. Moreover many *Escherichia* strains excrete antibiotic substances which suppress the growth of *Salmonella* (Levine and Tanimoto, 1954) and the latter may be eliminated, therefore, in mixed cultures containing the two organisms.

In the present paper a new selenite brilliant green medium is described in which *Escherichia* and *Proteus* will not develop and in which luxuriant growth of *Salmonella* occurs even when the inoculum consists of only one cell per ml of medium.

MATERIALS AND METHODS

A large number of modifications of selenite broth were tested and the medium which proved to be best had the following composition:

	Per cent
Peptone[1]	0.5
Yeast extract[1]	0.5
Mannitol	0.5
Sodium selenite	0.4
Sodium taurocholate[1]	0.1
Brilliant green[1]	0.0005
Phosphate buffer, pH 7.0	0.025 molar
Distilled water	

In the preparation of this medium, the first five ingredients were dissolved in somewhat less than the required amount of water and adjusted to pH 7.0 by the addition of a few drops of 5N HCl. The phosphate buffer and brilliant green were then added and the volume of the medium was adjusted to the required level with water.

The phosphate buffer was prepared by mixing appropriate quantities of 0.25M solutions of KH_2PO_4 and K_2HPO_4 to give a solution of pH 7.0 and this was added to the medium in a ratio of one part of buffer to 10 parts of medium.

The brilliant green had a dye content of 93 per cent.

[1] These are Difco products. Mention of these products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

One ml of a 0.1 per cent aqueous solution of the dye was added to each 200 ml of medium. Since the dye solutions lost considerable color on standing overnight in the laboratory, a fresh solution was prepared on each day that a new batch of medium was to be made.

The medium was tubed in 9-ml amounts and heated in flowing steam for 30 minutes prior to use.

The suspensions of *Salmonella* and other bacteria were prepared by growing the organisms on trypticase soy agar plates for one day at 35 C. The cells were washed from the plates with sterile distilled water, filtered through gauze, and stored in flasks in the refrigerator. The flasks contained glass beads to help disperse the cells. The numbers of viable cells in the suspensions remained fairly constant during storage for one to two months. For use as inoculum, a portion of the cell suspension was diluted with water to give approximately the required number of cells per ml, and the actual number of cells present was determined by plating. In order to simulate natural conditions where there are usually only a few *Salmonella* cells mixed with a larger number of other microorganisms, the inocula were adjusted so that approximately only one *Salmonella* cell and 100 cells of other bacteria were inoculated per ml of medium. One ml of inoculum was added to 9 ml of medium.

The cultures were incubated for about 18 hours at 35 C and then plated with trypticase soy agar to determine the fate of the inoculated cells. The tendency of the *Proteus* strains to swarm over the agar surface was effectively controlled by layering the poured and solidified plate with a small amount of the agar medium.

Additional details of methodology will be given at appropriate places in the text.

RESULTS

Some of the advantages and deficiencies of selenite F medium are illustrated by the data in table 1. The broth yields millions of salmonellae from inocula of 1 or 2 cells per ml. *Salmonella pullorum*, in contrast to the other strains, grows poorly but sufficiently to give rise to numerous colonies when streaked on solid media.

TABLE 1. *Growth of Salmonella and other bacteria in Selenite-F medium (BBL)*

Organism		Inoculum	Growth
		Cells per ml	*Cells per ml*
Salmonella oranienburg	200E	2	65,000,000
Salmonella typhimurium	TM-1	2	36,000,000
Salmonella anatis	5343	1	47,000,000
Salmonella pullorum	3083	1	88,000
Proteus vulgaris	P100	95	109,000,000
Proteus mirabilis	P112	102	20,000,000
Proteus rettgeri	P113	59	23,000,000
Escherichia coli	451B	292	3,500,000
Aerobacter cloacae	460	38	3,400,000

TABLE 2. *Growth of Salmonella and other bacteria in selenite brilliant green medium*

Organism		Inoculum	Growth
		Cells per ml	*Cells per ml*
Salmonella oranienburg	200E	1	310,000,000
Salmonella typhimurium	TM-1	1	170,000,000
Salmonella anatis	5343	2	490,000,000
Salmonella pullorum	3083	1	88,000
Proteus vulgaris	P100	116	0
Proteus mirabilis	P112	129	30
Proteus rettgeri	P113	94	0
Proteus morganii	B540	111	1,800
Escherichia coli	451B	131	0
Escherichia coli	456	192	20
Aerobacter cloacae	460	81	131,000
Streptococcus faecalis	9790	121	0
Alcaligenes faecalis	B170	163	0
Pseudomonas aeruginosa	9627	85	0

Strains of *Proteus*, *Escherichia*, and *Aerobacter*, however, also multiply readily in selenite F broth and give rise to millions of cells per ml of medium within 18 hours.

The selenite brilliant green medium also supports luxuriant growth of *Salmonella* from very small inocula (table 2). But in marked contrast to selenite F medium, the modified broth inhibits the development of strains of *Proteus* and *Escherichia*; only a few cells of the inocula can be recovered after incubation. The only exception is the strain of *Proteus morganii* which made slight but virtually insignificant growth. The inhibition of *Aerobacter cloacae*, although greater than that obtained in selenite F broth, is incomplete. The multiplication of *Streptococcus faecalis*, *Alcaligenes faecalis*, and *Pseudomonas aeruginosa* is completely repressed.

The ability of additional strains of bacteria to grow in the selenite brilliant green medium was tested by a simplified technique. The bacteria were grown for one day in broth. The *Salmonella* strains were diluted a thousand fold prior to inoculation by needle into the selenite medium, whereas the other strains were not diluted. All of the *Salmonella* strains grew abundantly in the selenite brilliant green medium. These included five strains of *S. pullorum*, four strains of *S. senftenberg*, two strains each of *S. meleagridis* and *S. anatis*, and one strain each of *S. paratyphi* B and *S. newport*. None of six strains of *E. coli* grew and the same was true of three strains of *P. vulgaris*, two strains of *P. morganii*, one strain of *Pseudomonas fluorescens* and also one strain each of the aerobic spore formers, *Bacillus brevis*, *B. cereus*, *B. mesentericus*, and *B. macerans*. In contrast, three of four strains of *Aerobacter aerogenes* grew.

The degree of inhibition of strains of *Proteus* and *Escherichia* in the selenite brilliant green broth is strikingly illustrated by the data in table 3 on the effect of size of inoculum on growth. An eight-fold increase in the number of cells of *S. oranienburg* inoculated did not

TABLE 3. *Effect of size of inoculum on growth in selenite brilliant green medium*

Organism		Inoculum	Growth
		Cells per ml	*Cells per ml*
Salmonella oranienburg	200E	1	590,000,000
		8	510,000,000
Salmonella pullorum	3083	1	230,000
		36	36,000,000
Proteus vulgaris	P100	11,000	120
		1,100,000	19,400
Escherichia coli	451B	14,300	270
		1,400,000	3,600

alter significantly the size of the final cell population but a 36-fold increase of *S. pullorum* raised the final population from 230,000 to 36,000,000 cells per ml. The most important aspect of the data in table 3, however, is the complete repression of the multiplication of *P. vulgaris* and *E. coli* even when large inocula of more than a million cells per ml of medium are used. The inoculated cells rapidly die in the unfavorable medium.

The inhibitory properties of the new medium are the sum of the activities of the selenite, brilliant green, and taurocholate contained in it. The effects of these three substances on growth, singly and in combination, are illustrated by the data in table 4. When none of these substances is in the medium, very large populations of *S. oranienburg, P. vulgaris and E. coli* are obtained. With all three organisms, the addition of selenite alone greatly reduces growth, brilliant green completely inhibits development, and taurocholate has little or no effect.

When both selenite and brilliant green are used, all growth is suppressed as is the case with brilliant green alone. With selenite plus taurocholate, less inhibition of *S. oranienburg* and *E. coli* and more inhibition of *P. vulgaris* occur than with selenite alone. But the most striking and useful results are obtained when brilliant green is used in combination with taurocholate. The latter eliminates the toxicity of the brilliant green for *S. oranienburg* but not for the other two organisms. The greater selectivity of the new medium over selenite F broth is due, therefore, primarily although not entirely, to its content of a balanced mixture of brilliant green and taurocholate. The further addition of selenite does not alter the selectivity of the medium, but decreases the *Salmonella* population. The reasons for retaining selenite in the modified medium will be discussed later.

The modified medium also differs from selenite F broth in that it contains mannitol in place of lactose. The reason for the use of lactose in selenite F broth was clearly stated by Leifson (1936):

"The lactose in the medium serves to maintain a uniform pH. When selenite is reduced by the growth of bacteria, alkali is produced and such increase in pH would lessen the toxicity of the selenite and result in overgrowth of extraneous bacteria. The acid produced by fermentation of the lactose by enterococci and to a lesser extent by colon bacilli serves to maintain a neutral or slightly decreased pH."

TABLE 4. *Effect of selenite, brilliant green and taurocholate on growth*

Addendum*	*Salmonella oranienburg*	*Proteus vulgaris*	*Escherichia coli*
	Cells per ml	*Cells per ml*	*Cells per ml*
None	1,800,000,000	400,000,000	1,700,000,000
Sodium selenite, 0.4%	22,000,000	100,000,000	390,000
Brilliant green, 0.0005%	0	0	0
Sodium taurocholate, 0.1%	1,100,000,000	400,000,000	1,100,000,000
Selenite + brilliant green	0 600,000,000	0	0
Selenite + taurocholate	1,400,000,000	26,000,000	930,000
Brilliant green + taurocholate	180,000,000	0	0
Selenite + brilliant green + taurocholate		0	0

* The basal medium contained peptone, yeast extract, mannitol and phosphate buffer.

Because lactose cannot be utilized by strains of *Salmonella*, it favors unduly the growth of the competing bacteria. Theoretically it would be best to use a sugar which is fermented only by *Salmonella*. This would serve to favor the growth of only *Salmonella* as well as to help maintain the proper pH. But since a carbohydrate with such desirable selective properties is not known, it seemed best to substitute for lactose some sugar which can, at least, also be fermented by *Salmonella* so that their growth also can be stimulated. Mannitol was selected because it is fermented by virtually all strains of *Salmonella*. Although most strains of *Escherichia* ferment mannitol, it is unavailable to all but one species of *Proteus*, namely *P. rettgeri*, and therefore the growth of most *Proteus* strains is not helped by this carbohydrate.

The amount of phosphate in the medium is of considerable importance. All of the test organisms failed to grow when phosphate was omitted. This was somewhat surprising because the peptone and yeast extract in the medium can be expected, usually, to supply sufficient phosphate, as an impurity, for microbial growth. It seems likely that the other ingredients of the medium, particularly the inhibitory compounds selenite and brilliant green, increase the phosphate requirements above the normal level. Conversely, a relatively high concentration of phosphate, 0.1 M, stimulates the growth of *Proteus* and inhibits partially some strains of *Salmonella*. The phosphate concentration finally adopted, 0.025 M, was best for the dual purpose of stimulating *Salmonella* and inhibiting *Proteus*. Recently

North and Bartram (1953) have shown that growth of *Salmonella* in selenite F broth is improved when the phosphate concentration is reduced from 1.0 to 0.25 per cent.

They noted, also, that selenite F broth prepared from some batches of peptone did not support the growth of *Salmonella* and that the addition of small amounts of yeast extract corrected this unsatisfactory condition. The presence of both peptone and yeast extract in our medium should serve to avoid this difficulty.

Discussion

It has been established that for the isolation of *Salmonella* from natural sources preliminary enrichment in suitable media leads to a greater number of isolations than direct plating procedures. During the past half century, a large variety of enrichment media for *Salmonella* have been developed and used, each possessing certain advantages and disadvantages (Mallman *et al.*, 1942; Williams Smith, 1952; Banwart and Ayres, 1953). The special merit of the selenite brilliant green medium described in the present paper is its marked ability to suppress the growth of strains of *Escherichia* and *Proteus*. Moreover, the medium contains a balanced mixture of three inhibitory compounds selenite, brilliant green, and taurocholate which are active against a large variety of gram positive and gram negative bacteria. This results in a highly selective medium, but one which nevertheless permits abundant growth of *Salmonella* from very small inocula.

One disadvantage of brilliant green is the tendency of the dye to lose its selective properties in the presence of high concentrations of organic matter (Browning *et al.*, 1913; Krumwiede and Pratt, 1914; Stark and Curtis, 1936). This becomes important whenever large amounts of organic matter, in the form of test material, are added to the medium. A similar situation exists in the case of selenite (Leifson, 1936). Although the selenite does not seem to be essential for our medium, it has been retained to protect the selective action of the medium, as much as possible, against the neutralizing effect of added organic matter. Despite this precaution, the addition of 10 per cent of liquid whole egg to the medium reduces its ability to suppress the growth of *Escherichia* and especially *Proteus*. This effect of egg is a general phenomenon which occurs with many of the commonly employed *Salmonella* enrichment media (Hurley and Ayres, 1953) and is now under investigation.

Our data are derived from studies with pure cultures and it is possible therefore that different results might be obtained when isolations of *Salmonella* from natural materials are attempted. But there is reason to believe that the advantages of the selenite brilliant green medium will be retained in practice. The addition of brilliant green and bile salts to tetrathionate medium resulted in a considerable increase in the number of isolations of *Salmonella* (Kauffman, 1935) and therefore one may expect that similar improvement will occur on the addition of brilliant green and bile salts to the selenite medium. The other modifications of the selenite medium, that is, use of both peptone and yeast extract, substitution of mannitol for lactose and regulation of the phosphate concentration should also prove helpful in the isolation of *Salmonella*.

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Summary

A selenite brilliant green enrichment medium for the isolation of *Salmonella* is described. It is more effective in preventing the growth of strains of *Escherichia* and *Proteus* than the commonly used selenite F broth. Although the new medium is highly selective, it supports abundant growth of *Salmonella* from very small inocula.

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