A new approach to the preparation of bicarbonate solution for use in virus isolation media

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The preparation of bicarbonate solution in small sterile aliquots for addition to culture media as a buffering agent is standard practice in many laboratories. It is necessary to pre-gas this solution with carbon dioxide in order to ensure a final pH of 7.4 and adequate buffering in the final medium. It is difficult and time-consuming to obtain a sufficient concentration of carbon dioxide in the solution, particularly as autoclaving the aliquots even with tight stoppering causes some loss of carbon dioxide. The need for a new and expensive regulator for the carbon dioxide cylinder caused us to look for a more efficient method of gassing. An ordinary 1.8 litre Sparklets soda syphon was found to be a cheaper alternative, to save a considerable amount of technician time, and to be more effective, giving an increased shelf-life to the aliquots.

Material and methods

BICARBONATE SOLUTION

Sodium hydrogen carbonate 88 g
0.2% Phenol red1 100 ml
Deionised water 1900 ml

The sodium hydrogen carbonate and 0.2% phenol red are placed in a flask and made up to 2 litres with deionised water. This is used to fill the 1.8 litre Sparklets soda syphon, which is then gassed using two Sparklets carbon dioxide bulbs, and the contents are shaken vigorously for two 

0.2% phenol red solution is made by dissolving 100 mg of phenol red in 5 ml 0.05 molar sodium hydroxide and diluting to 50 ml with deionised water.

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Discussion

above, streptomycin. The culture of the Coxsackie B cell lines requires some control of carbon dioxide tension in the gas phase and in the medium (Paul, 1973). Harris (1954) suggests that for the growth of some cell types the bicarbonate ion is essential. It is standard practice, therefore, to add bicarbonate solution to tissue culture media. Sodium bicarbonate converts to sodium carbonate with the release of carbon dioxide, particularly when autoclaved, and it is necessary to saturate the solution with carbon dioxide to convert all the carbonate to bicarbonate and keep it in that state. Hitherto we have saturated our bicarbonate solution with carbon dioxide by bubbling carbon dioxide through it in two stages, firstly in bulk and secondly when it has been dispensed into 10 ml aliquots in universal bottles. This method results in considerable loss of medium through excessive frothing and is very time-consuming and tedious. The first stage takes 60 minutes and needs constant attention because of frothing, and the second stage takes 30 seconds/bottle (a further 90 minutes for 180 aliquots). Because of the limited amount of carbon dioxide that can be dissolved in this way we found that the shelf-life of these solutions, even in tightly stoppered bottles, rarely exceeds one month. Using the soda syphon modification we can gas 180 aliquots in four minutes and, because of the extended shelf-life, batches need be prepared only infrequently and none has had to be discarded, thereby eliminating wastage of material and labour. We have shown that a solution produced by our method is easier and quicker to prepare, has a superior shelf-life, and is satisfactory in use.

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References