Modification of the Agar Sausage of Ten Cate for Bacteriological Control

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SUMMARY

The manufacture and use of a disposable plastic syringe as a container for a column of solid culture medium, instead of a plastic sausage casing, is described. The method of sampling and its usefulness in the supervision of the hygiene in hospital kitchens or any food-manufacturing establishment, is discussed. Particular reference is made to its use in determining the bacterial load of a surface in contact with food, and its value as a means of educating staff in the necessity for the thorough cleaning of all working surfaces. A standard of evaluation of the bacterial counts obtained by this method of sampling is suggested.

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Control of the hygiene in food-manufacturing plants and kitchens by means of the agar sausage, as described by Ten Cate[1] in Europe and Louw[2] in South Africa, has proved most useful. It is used for the bacteriological sampling of surfaces of equipment and of foods. The results obtained are no more variable than those obtained by swabbing techniques[3] and have the considerable advantages of requiring a minimum of apparatus and low cost. The whole process can be carried out by lay staff after one or two demonstrations. Most important of all is its usefulness as a means of educating kitchen or food factory personnel.

Briefly, the agar sausage consists of a length of plastic sausage casing filled with agar media. Both ends are tied and sealed. When testing a surface or a food, the end of the sausage is cut off and the agar column is brought into contact with the area to be tested. A slice about 0,5 cm in thickness is cut off with a sterile knife, and placed in a Petri dish with the exposed surface uppermost. Four slices are usually placed in one Petri dish, which is then incubated overnight at 37°C. It is advisable to place one slice, which has not been in contact with the specimens being examined, in at least every second dish, to act as a control for sterility.

After fairly extensive experience of its use in food plants and in hospital kitchens this method was found to have the following disadvantages:

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(a) only a full roll of plastic sausage casing could be bought—this was expensive and wasteful;
(b) manufacture of the agar sausage was time-consuming and troublesome;
(c) the sausages could not reach far enough into machines to test their cleanliness;
(d) the sausage was wasteful, in that once opened, the whole had to be used;
(e) it was often not possible to use the last 4 - 6 cm of the sausage, since it slipped out of its casing and became contaminated.

To overcome all the above disadvantages a modified 50-ml disposable plastic syringe was used as a container for the agar column in place of the plastic sausage casing (Fig. 1).

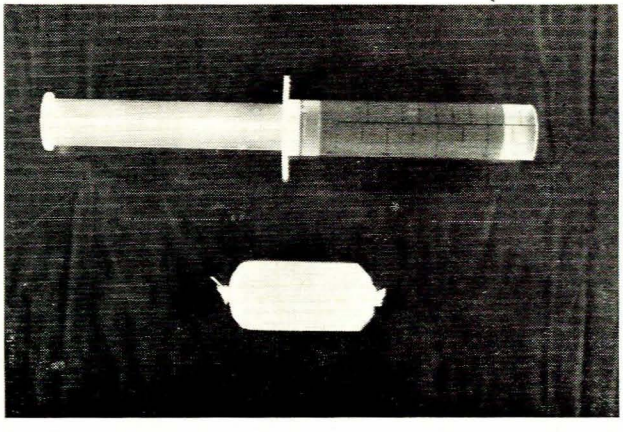

Fig. 1. Top: **Agar column in plastic syringe.** Bottom: **Agar sausage.**

MATERIALS AND METHODS

Preparation of Agar Column

Bacteriological medium (either Hartley's nutrient agar medium or MacConkey's medium) is prepared according to the manufacturer's instructions. To obtain a firm consistency the agar content of the medium is increased to 30 g/litre (3%).

A sterile, disposable 50-ml plastic syringe is removed from its sterile paper packet by tearing off one end, and the nozzle end of the syringe is cut off with a heated sterile knife blade, after easing out the piston for almost the full length of the barrel.

The syringe is filled with the hot, sterilised culture medium to within 1 cm of the end. It is again covered with its sterile paper wrapper, the open end of which is taped round the barrel of the syringe. The syringe is then stood, end up, in a refrigerator until the medium has set. When set, the whole paper-covered syringe is placed in a plastic bag which is tightly closed and kept in a refrigerator until used. The loaded syringes can be stored in this way for at least 3 months.

One syringe out of every ten is incubated for 48 hours and examined for sterility.

Sampling Procedure

The plastic bag and paper packet are removed.

The plunger of the syringe is depressed to extrude about 0,75 cm of the agar column, and the flat end of the column is applied firmly onto the surface to be tested.

A sharp, broad-bladed knife (No. 24 Bard-Parker blade), previously sterilised in a flame, is used to cut off a slice of agar about 0,5 cm thick. This slice is placed in a sterile Petri dish with the exposed side uppermost and incubated for 18 - 24 hours at 37°C, whereupon the surface is examined for colonies.

Evaluation

It must be remembered that this method only determines the number of the bacteria that adhered to the surface of the agar column and that have grown on the medium, at the temperature used for incubation. It is by no means a method of counting all the bacteria on the area tested. The results should therefore be recorded only approximately, as follows: colony count: nil (0), 1 - 5 (+), 5 - 10 (++) 10 - 20 (+++), > 20 (++++). A count of 0 - 5 colonies is regarded as satisfactory.

Pressure of the agar column on a surface does not pick up all the bacteria on that area. Retesting of the same area does, however, show a marked fall in the number of colonies grown with each subsequent tes Experiments carried out show that there is no regul progressive fall with each test in the number of colonie grown. A heavily contaminated surface giving a markedl 'too numerous to count' (TN) result showed the followin counts for subsequent tests taken from the same are at half-minute intervals: TN 15, 12, and 4 colonies.

This method of testing is therefore neither more nc less accurate than the standard swabbing method whic also gives irregular results. Counts can only be show as approximate numbers. They merely indicate the degre of contamination which in turn is an indication of th efficiency of cleansing and disinfection of the surfac tested (Fig. 2.)

DISCUSSION

The use of the plastic syringe for holding and extrudin the column of agar has proved to be a definite improve ment over the use of a plastic sausage casing, as it i both easier to handle and to prepare and is more econo mical because almost the entire length of the column ir the syringe can be used. An average of 15 tests pe syringe can be carried out. Different media can be used but it has been found that nutrient agar is the most useful general-purpose medium for most surfaces. For heavily contaminated surfaces, particularly those which have come into contact with meat, a selective medium such as MacConkey's medium also proved most useful, as it is possible to obtain an idea of the type of organisms present. From both media, colonies can be picked off and identified.

There is no doubt, however, that the main advantage of this method is its educational value. Visual demonstrations to kitchen or factory staff, of the marked difference of bacterial growth from hands before and after washing, between the ordinary 'cleaned' working surfaces and the same surfaces after thorough cleansing and disinfection, etc., has brought about a great improvement in the workers' attitude to the existing hygienic practice and made them understand why thoroughness is necessary.

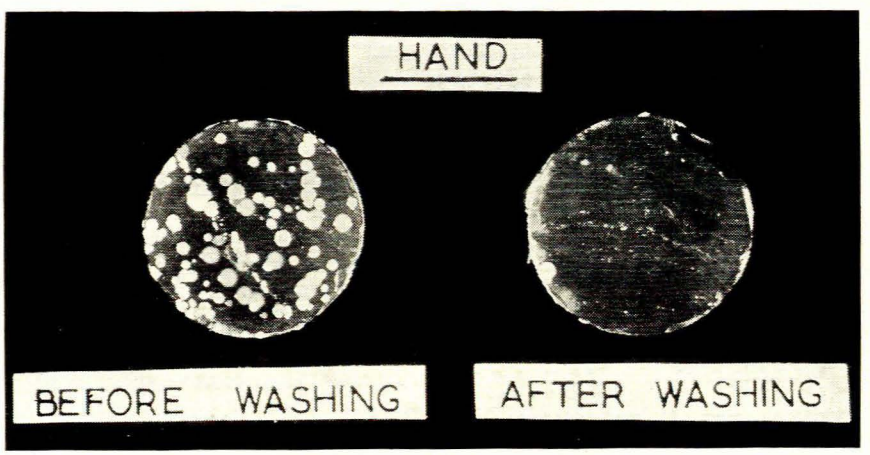

Fig. 2. Control of hand hygiene. Bacterial growth on agar discs.

In addition, the supervising staff in hospital kitchens have been shown how to carry out the test and have found it most useful in the supervision of the cleaning of utensils, equipment and working surfaces.

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Diabetogenic Drugs in the Vervet Monkey

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SUMMARY

Alloxan and streptozotocin were used to cause beta cell lysis in vervet monkeys used as recipient models for pancreatic allografts.

Tests were performed on these animals to evaluate the effect of the drugs on carbohydrate metabolism. Streptozotocin is preferred as the drug of choice in creating a non-pancreatectomised hyperglycaemic recipient for pancreatic allografting.

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The first vascularised allograft was performed on the dog in 1957.[1] Most of the subsequent work in this field has been carried out in this species.[2,3] Kelly *et al.*[4] and Lillehei and others[5] have carried out simultaneous renal and pancreatic allografting on patients with juvenile onset diabetes and diabetic nephropathy. Marks *et al.*[6] have indicated the importance of late islet cell deficiency as a common cause of death in intractable chronic pancreatitis. The final role of vascularised pancreatic allografts remains to be assessed, but initial experience has suggested that the method can be employed to reverse at least some of the effects of the islet deficiency. The anatomy of the pancreas is similar in man and in the other higher primates. The vervet monkey (*Cercopithecus pygerythrus* F. Cuvier) has been chosen as a non-human model because it is readily available in Natal and has been the subject of immunological study in this region. The viability of a pancreatic allograft may be assessed by the capacity of the graft to produce insulin. Total pancreatectomy before grafting is laborious, hazardous and removes all exocrine pancreatic tissue. Selective destruction of the beta cells of the islets of the recipient has therefore been preferred as a method of providing a 'diabetic' model in a small non-human primate.

Two drugs are available for this purpose. The diabetogenic action of alloxan in rats has been reported.[7,8] Streptozotocin has been evaluated in a wider range of animals, including non-human primates.[9-11] Neither has been evaluated in the vervet monkey. This study has been designed to evaluate their diabetogenic action in this species. Plasma glucose levels in fasting animals, urine volumes, pH, and urinary glucose levels were determined, and glucose tolerance tests have been carried out. The glucose tolerance tests were carried out as subdiabetic animals have normal fasting glucose levels but show impaired carbohydrate metabolism when challenged with a glucose load.

MATERIALS AND METHODS

Animals and Diet

Ten healthy adult vervet monkeys of mass 2,0 kg to 5,15 kg were housed in individual metabolic cages. For a month before the tests they were given a standardised diet consisting of approximately 70% carbohydrate and 30% protein. Enough fresh fruit and vegetables were included to ensure an adequate supply of mineral salts and

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