A simple device for washing out radioimmunoassay tubes

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The use of disposable polystyrene tubes for radioimmunoassay is now widespread. Washing out such tubes before disposal is tedious and time-consuming, especially if they contain dense charcoal or double-antibody pellets. In this laboratory the simple device described below has much reduced the time and tedium involved in this task. It was constructed in a few minutes for a cost of a few pence.

A hypodermic needle (19 g $\times$ 2 inches) is fixed to a length of plastic or rubber tubing (about 5 mm outside diameter, 4 mm bore) either by tying or simply by threading as shown in the figure. The plastic tube is connected to a pump which preferably discharges directly into a sink outlet or overflow. The needle is connected to a tap. Part of a disposable 1 ml-syringe makes a convenient adaptor. The apparatus is fixed in a clamp at a comfortable working height above a sink. For use water is passed through the needle (about 100-120 ml/min) and the pump turned on. Tubes to be washed are uncapped, held as shown over the apparatus for a few seconds, and discarded, conveniently into a plastic disposal bag fixed to the edge of the sink or bench. Obstinate

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Fig Tube-washing device with tube in position. For details see text.

pellets are rapidly dislodged and sucked away by turning the tube round to break them up with the point of the needle.

Typically about 10 tubes are washed per minute. At this rate activity is reduced from 5000 counts/min to 40 counts/min above background (about 3×10^{-5} μ Ci per tube) even in tubes with dense charcoal pellets. (These are tubes used in an insulin assay with 50 pg/tube 125 I-labelled insulin). More than 60000 tubes could thus be discarded per day before exceeding the current disposal limit in this laboratory for solid waste of 2 μ Ci per day per room.

Letters to the Editor

Concentration of Protein Solutions

When aqueous solutions of electrolytes are cooled, ice crystals are formed which are fundamentally the same as those formed in pure water; as freezing proceeds solute concentration occurs in the interstices and, theoretically at least, can eventually achieve a complete separation of vitreous water and dehydrated solute.¹

Since dehydration by freeze-drying has often been used as a means of concentrating protein solutions, for example in csf and urine, but is time consuming and

tends to cause denaturation, the possibility was considered of partial freezing, at a temperature high enough to minimize denaturation effects but low enough to separate water as ice and thus effect a solute concentration.

The temperature chosen was above the eutectic temperature of sodium chloride (-21°C) at which temperature the denaturation of β -lipoprotein in a rising electrolyte concentration rises sharply. Since the average deep freeze runs at a temperature of -17°C to -20°C the following technique was carried out using a domestic deep freeze operated at a temperature of -18°C .

Two millilitres of the fluid to be

concentrated is placed in a plastic tube (polystyrene, 12 mm diameter) in a rack in the deep freeze. When completely frozen (one to two hours) the tube is held firmly in the horizontal plane and the first melted drop of fluid is captured in a drawn-out glass capillary. It is important not to permit the melting of more than about 20 μ l of the frozen contents. The capillary is then used to apply the concentrated protein solution to the electrophoretic strip and, if required, the remainder of the capillary sample can be used in a micro-assay for total protein. For freezing the tube is held vertically in a metal rack ensuring one free space on each side of the tube. In the thawing technique the tube is

held in the closed fist rather than by the fingers, to obtain as even as possible an application of heat to the tube, keeping sufficient clear view of the meniscus to pick up the first drop of melt with the capillary; the intention is to permit sufficient heat energy from the hand to penetrate the plastics tube only to melt the outer surface of the ice mass. The dehydrated solute lying at the interface of ice mass and tube is redissolved in the small quantity of water which is permitted to result.

Methods for Measuring Serum Gentamicin Concentrations

In two papers (*J. clin. Pathol.*, 1974, 27, 447-451, 452-456) Phillips *et al* and Ten Krooden and Darrell have compared three methods for measuring serum gentamicin concentrations. Both state that the urease method (Noone, Pattison, and Samson, 1971; Noone, Pattison, and Slack, 1972) is less accurate than either the 18-hour plate assay or the adenylase method of Smith, Van Otto, and Smith (1972). It was insufficiently emphasized that this inaccuracy is not an intrinsic feature of the urease method *per se* but probably the result of inexperience with the method.

Although we too have compared the three methods, we have not sought to publish the results because we consider that, since we do not have equivalent experience and expertise with all three methods, there could not be a valid comparison. Surely the only way to compare these methods for their intrinsic accuracy and reproducibility is by comparing the results obtained by workers equally experienced in the use of the individual methods. We have had a great deal of experience with the urease method and have elucidated its mode of action (Pattison *et al*, 1974).

Our own evaluation of the urease

The technique has been in use in this laboratory for two years and has given excellent results, comparable with osmotic methods of concentration with apparently less denaturation. In this connexion it is interesting to note that we have not seen the 'tau' fraction on csf electrophoresis using this means of concentration,² although comparison of serum before and after dilution and reconcentration sometimes indicates diminution in β -globulin levels.

method (in press) shows that it can produce results as consistently accurate as the best performed plate assays (table). From the data of Ten Krooden and Darrell, it is apparent that our results are on the whole better than the adenylase method in their hands (table). In this study the urease assays were performed by two junior technicians and one qualified technician (each doing eight assays at each nominal concentration). None of them had had more than six months' experience with the method.

Ten Krooden and Darrell correctly stress the necessity of obtaining results rapidly in order to provide the data required for accurately controlling gentamicin therapy in seriously ill patients. However, both these workers and Phillips *et al* have used a plate diffusion assay involving 18 hours of incubation in their comparisons. We know of no data that are available to compare the urease and 'rapid' plate assay method (incubation period four to six hours). Nor do we know of any extensive statistical comparison between rapid plate assays and overnight plate assays.

Therefore, it would seem that the only methods which will provide results rapidly enough for clinical use and which have been subjected to some statistical validation are the urease and adenylase methods.

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References

- ¹Smith, A. U. (1961). *Biological Effects of Freezing and Supercooling*, Ch. 11. Arnold, London.
²Henry, R. J., *et al* (1974). *Clinical Chemistry, Principles and Techniques*, 2nd ed., p. 445. Harper and Row, New York and London.

Since both methods can be of equivalent accuracy the decision as to which method to use must be based on other factors. The urease assay is more rapid and less expensive than the adenylase method; and the salt-free variation (Noone *et al*, 1972) requires only 0.3 ml of serum. The only technical skill required is that of pipetting. Because of the small volumes involved this must be accurate. We have found that Oxford and Ependorff automatic pipettes are satisfactory providing care is taken to use them properly (in particular to avoid bubbles in the tip).

The only expense is the need for a pH meter, reading to two decimal places (about £200-£250). That used by Phillips *et al* is stated as reading to one decimal place only, which greatly reduces the potential for accuracy.

The urease method can be organized easily in routine, clinical laboratory practice. Assay tubes, containing urea medium and gentamicin standards only, are made up on Monday morning to provide sufficient sets for a week's tests. This will take about one hour at the most. The sets are stored at 4°C or frozen until needed when (after thawing) test and pool serum and the proteus inoculum are added. This takes five to 10 minutes at the most. The proteus is subcultured each day into fresh Todd Hewitt broth

Expected Concentration	Number of Assays	Mean	Mean Percentage Error	Standard Deviation	Mean + 2 SD of Percentage Error
<i>Ten Krooden and Darrell</i>					
3.0	8	2.7	10.0	0.47	45.0
5.0	8	4.97	0.6	0.71	29.2
6.5	8	7.30	12.0	0.51	26.0
12.0	8	11.61	3.0	0.72	15.4
<i>Noone, Pattison, and Garfield Davies</i>					
4.0	24	4.17	4.0	0.43	24.6
8.0	24	8.18	2.0	0.66	18.2
12.0	24	12.00	0	0.99	17.0

Table