REFRIGERATED TRANSPORT OF VIRUS SPECIMENS

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Introduction

SPECIMENS for virus isolation being transmitted on journeys of more than an hour or two require to be kept cold. To accomplish this, refrigerants such as water ice (wet ice) or solid carbon dioxide (dry ice) are employed, the specimen being placed in a vacuum flask or other insulated container together with wet or dry ice. Wet ice generally represents a poor substitute for dry ice but is often used when the latter is not available. Nearly all virological specimens frozen in dry ice remain in a condition suitable for examination for very long periods. Indeed if the dry ice is replenished regularly so that the temperature of the specimen does not rise, and provided that gaseous carbon dioxide is not allowed to reach the specimen, storage for many months is possible.

As more clinicians become familiar with the facilities available for the diagnosis of virus illness so the number of specimens requiring refrigerated transport can be expected to increase. The average individual consigning such a specimen probably has little idea how much refrigerant is required for a journey of given duration or how long a flask filled with refrigerant will remain cold. To provide quantitative data on such points the experiments reported here were carried out. Because dry ice is not always available, a series of experiments were performed with salt-wet ice freezing mixtures, to see if this might provide an alternative in case of necessity. In addition, because of the fragile nature of the vacuum flask a prototype box insulated with expanded polystyrene was tested to see if it could replace the flask.

Materials and Methods

Flasks

A series of 8 of the standard military issue vacuum flasks (Jars, vacuum, one quart) were chosen at random. These were tested comparatively for their insulating qualities under identical conditions, and on the results of the tests the best and worst flasks from among the 8 were chosen for more detailed study. The flasks used have an exact counterpart which is commercially available as the "Thermos" brand model No. 707.

Insulated box

The expanded polystyrene used in the construction of the box was obtained in sheets 1 inch thick as supplied for insulation of domestic water tanks, etc. This thickness was used so that a box made with a double layer of it had a 2 inch insulating wall in all directions with overlapping joints. The outside of the box so constructed was covered with quarter-inch plywood to provide a rigid protective skin. The box measured 10 x 7½ inches externally, and had an internal space measuring 5 x 3 x 3 inches. Ordinary carpenter's glue was used in its construction, though a special cement for use with the plastic material is available. The essential structural details can be deduced from Plates 1 and 2.

Plates 1 and 2. Views of the expanded polystyrene insulated box, to show essential constructional details.

The cost of the materials used in making this box was well under ten shillings.

Temperature recording

Temperatures were recorded by means of a continuous writing device actuated by a platinum resistance thermometer. The probe of the thermometer was surrounded by 3 quarter-ounce ("bijou") bottles each containing 3 ml. of water, held together by an elastic band. This was the standard load employed throughout the experiment. The probe plus standard load was placed in the flask or box, and was surrounded by the refrigerant under test. The container was then closed, the wire to the probe being led out through the cork bung of the flask or directly through the side of the insulated box. The flask or box was then placed at the ambient temperature concerned. The experiment was continued until the temperature reached 0°C , and the elapsed time was measured from the temperature trace. In each case the standard load and container were chilled to 4°C in a refrigerator before the test was started.

Three ambient temperatures were employed, namely 37 , 25 and 2°C . Charges of 200, 300 and 400 gm. of dry ice were tested at each temperature, and each test was repeated with the best and worst flask, and with the insulated box. The salt-wet ice mixture employed was in the ratio of 1 part salt to 3 parts ice by weight. The ice used consisted of slightly crushed cubes from a domestic refrigerator. It was weighed in a funnel to avoid including water, was added to the pre-chilled flask containing the standard load and the correct amount of salt was then poured over the whole. Immediately after this the flask

was closed and temperature and time recording commenced. In this part of the experiment only the worst flask was used, with varying charges of the freezing mixture at various ambient temperatures.

Results

These are presented in tabular and graphic form. The results of the comparative experiments with 8 flasks are shown in the Table, from which it can be seen that the worst

Table
Times taken by a random selection of vacuum flasks each charged with 200 gm. dry ice to reach 0°C, at an ambient temperature of 37°C.

Flask No.	Time (hrs)
1	19.2
2	19.3
3	19.0
4	19.2
5	19.4
6	17.5
7	20.5
8	19.3

flask was number 6 and the best number 7. These two flasks were used throughout the remainder of the experiments. Figure 1 compares the general form of the warming-up curves of a flask containing in turn dry ice and a salt-wet ice freezing mixture, and of the insulated box with dry ice. Figure 2 compares the duration of the warming-up period in

Figure 1.

The general form of warming-up curves in a flask containing successively a salt-wet ice freezing mixture and dry ice, and the insulated box containing dry ice, all at 37°C. ambient temperature.

Figure 2.

A comparison of warming-up periods to 0°C. in 2 flasks containing varying charges of dry ice at 3 different ambient temperatures.

the worst and best flasks when each contained 200, 300 and 400 gm. dry ice, at 37, 25 and 2°C respectively. Figure 3 presents the same information, but using salt-wet ice mixtures

in the ratios 65:200, 100:300 and 130:400 gm. of each, respectively. A series using the freezing mixture at 2°C was not done as excessively long warming-up periods elapsed at this temperature. The worst flask only (No. 6) was used in the experiments with the freezing mixtures. Figure 4 gives detailed results of warming-up times at different ambient temperatures with the 3 standard charges of dry ice in the insulated box.

Figure 3.

A comparison of warming-up periods to 0°C. in a flask containing varying charges of salt-wet ice freezing mixture at 2 different ambient temperatures.

Figure 4.

A comparison of warming-up periods to 0°C. in an insulated box containing varying charges of dry ice at 3 different ambient temperatures.

Discussion

The 3 temperatures chosen provide approximations on the high (and therefore safe) side to tropical, temperate, and arctic conditions. By using the appropriate graph it is possible to calculate the amount of refrigerant needed to provide cover for a journey of known duration. By using the curve for the worst flask in each case a safety factor is introduced, allowing a small excess of refrigerant over the required amount. In addition, those dispatching specimens are recommended to allow an additional 25 per cent safety margin in case of accident or unscheduled delay in transit. The use of salt-wet ice freezing mixtures for transmission of virus specimens may be advised where dry ice is unobtainable. The lowest temperature achieved, -20°C, although better than nothing, is undoubtedly injurious, because initial freezing occurs at or about the eutectic point of many biological systems. This technique should thus only be employed as a last resort.

The insulated box, although inferior to a flask, can almost certainly find a place in the transportation of specimens over shorter distances, as for example within the U.K. by passenger train. Here its cheapness and virtual indestructibility must make it the container of choice. It would also find a place in the collection of specimens from the patient's bedside, or abroad for use between the small peripheral laboratory and a larger laboratory prior to final dispatch by air. Here again its more rugged construction would allow it to be carried safely during transport over rough terrain without fear of breakage.

Summary

Experiments are reported which establish the period during which refrigeration is maintained in vacuum flasks containing different amounts of dry ice and salt-wet ice freezing mixtures under various conditions. A prototype box insulated with expanded polystyrene was tested similarly, and it was established that such a container would be useful where shorter periods of refrigeration are required. Data are given for calculating the amount of refrigerant required for a standard load in various conditions.