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Dear Richard

HEAT INACTIVATION OF HTLV III

Following our recent discussion of this subject, I am writing to give you the details of the heat treatment process we have developed for our clotting factors, Basically, for FVIII, this involves the manufacture of a more purified product which is then stabilised with sorbitol and glycine. In this stabilisation mixture, FVIII can happily be pasteurised at 60°C for 10 hours with fairly low losses in FVIII activity. Using Vaccinia as a model for virus inactivation, we found that our stabilisation solution also resulted in significant stabilisation of the virus, as follows:-

SOLUTION	REDUCTION OF INFECTIVITY AT 60°C
PBS or Caprylate stabilised albumin	Entire inoculum ($<10^8$ PFU/ml) inactivated within 30 minutes
Stabilised FVIII	10^{2-5} - 10^4 log reduction in infectivity

On the basis of these findings and studies of the Kinetics of Vaccinia inactivation over different times and at different temperatures, we have developed more severe heating conditions of 16.5 hours at 60°C followed by 20 minutes at 70°C. This consistently inactivates our challenge inoculum of 10^3 PFU/ml and will also inactivate 10^7 PFU/ml of Herpes simplex and 10^7 PFU/ml of Polio type 2. Strangely, mumps virus is significantly stabilised in our mixture and even with our severe heating conditions we only a achieve a 10^3 - 10^4 log reduction of mumps infectivity. Unfortunately, we cannot achieve more severe heating conditions without severe yield penalties so we must live with the possibility that the viruses we wish to inactivate such as Hepatitis B, NANB will be similarly stabilised. However, Behringwerke using a sucrose stabilised product heated at 60°C for 10 hours have demonstrated inactivation of Hepatitis viruses using a chimp model and in our hands sucrose also stabilised viruses such as vaccinia and mumps.

It/

It is our intention to extend our range of model viruses in the near future to include Rous Sarcoma Virus and SV_{40}. It would, however, be of supreme relevance if we could demonstrate inactivation of HTLV III. To this end I would propose this relatively simple protocol:

1. We ship down some stabilised FVIII containing extra stabilisers to accommodate your virus inoculum volume.

2. Add virus to FVIII and leave at room temperature for 10 minutes or so to permit virus stabilisation - Sample.

3. Add to water bath set at 60°C ± 0.5°C, monitor temperature in second bottle + hold at 60°C for 16.5 hours - Sample.

4. Add to second water bath set at 70°C ± 0.5°C, allow mixture to achieve 69.5°C and then hold for 20 minutes - Sample.

When assaying for infectivity it is noteworthy that the solution has a very high sugar content (65% w/v) and some predilution is usually necessary to reduce the osmotic pressure on cell cultures. In our tissue culture systems we start infectivity assays at a 1:5 dilution for this reason.

I hope that you will be able to manage an assessment along these lines and look forward to hearing from you.

With Kind Regards

Yours sincerely

Bruce Cuthbertson, PhD
Microbiologist