# FVIII SAFETY ACTION GROUP

Meeting held on 23rd June 1982 at SNBTS Headquarters Unit Laboratory, 2 Forrest Road, Edinburgh.

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Protocol for infectivity model in the tamerin Saguinus labiatus using putative human non-A, non-B hepatitis virus.

## SUMMARY

The work is divided into three stages which should form self contained sub-projects to allow e.g. separate funding, redesign of experiments and/or premature termination. These stages are:

(i) Validation of infective source material

(ii) Infectivity titration

(iii) Inactivation titration.

The total period of time required may be up to 2 years, but is unlikely to be less than one year. The number of animals estimated for the work is nine, but some reuse of individuals may be possible.

The target for this work is three logs reduction in infectivity.

(i) Validation of infective material:

A single batch of material chosen either from commercial FVIII concentrates or an implicated blood donor will be freeze-dried in 1 ml vials by DSP. At least 40 vials will be prepared at one time.

One ml of this material will be reconstituted and injected intravenously into a tamerin and the blood will be taken at weekly intervals for ALT assays, preferably to be done locally by CPHLS. Sufficient sterile sample (1 ml) should be taken to provide 0.2 ml serum for ALT and 0.3 ml spare sterile aliquot for repeat ALT or other laboratory investigation or re-passage in tamerins. The animal inoculated should be followed for one month and if no significant elevation of ALT is seen, a second animal should be inoculated and both followed for a further month. If no ALT elevation is seen in the second animal, a third animal should be inoculated and followed for a further month. If no infectivity is documented, the same animals could be re-used in a repeat study with a second batch of material.

Total time required 5 months, total number of animals required 3.

(ii) Titration of infective material:

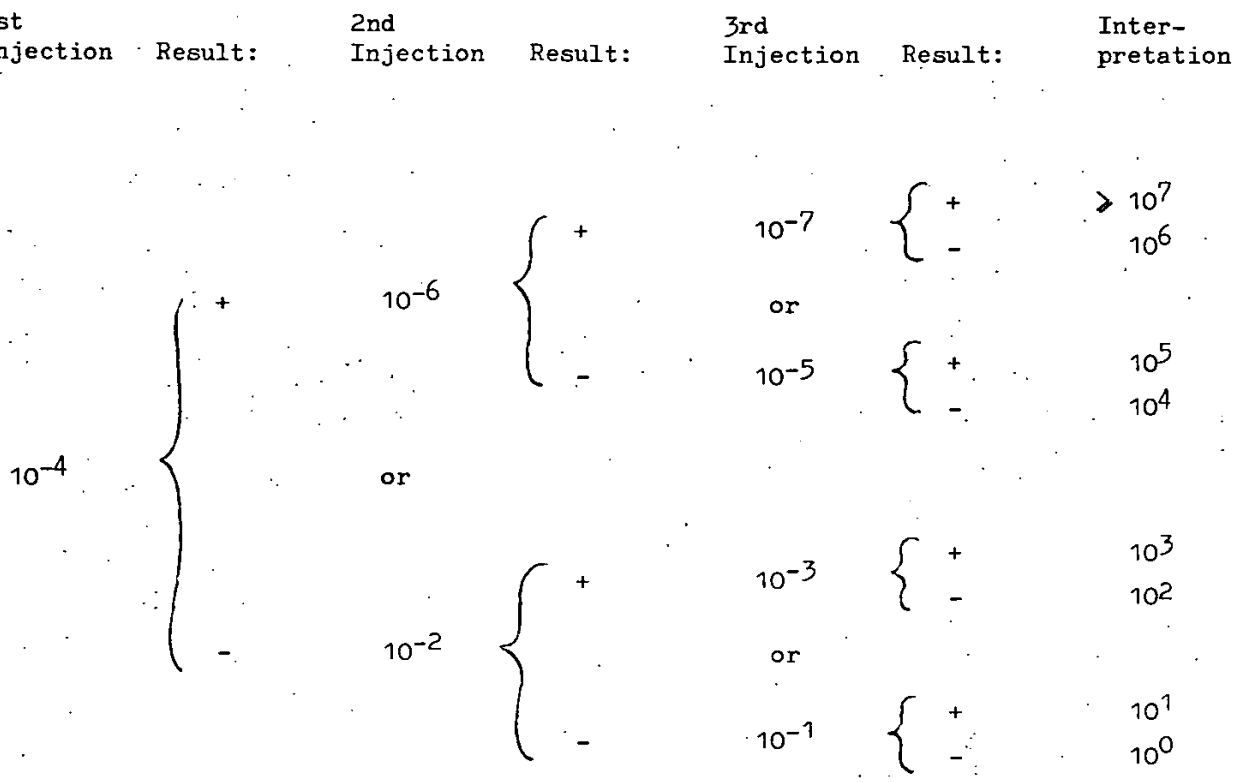
Exact design depends on results of above study but, for reasons of avoiding immunisation and anaphylaxis by human plasma proteins, it would seem desirable to titre positive serum from previously infected tamerins. Problems may arise with either insufficient volume of material and/or uncertainty as to when animals are viraemic. Assuming adaptation of human material, both titre and infectivity should improve using re-passaged tamerin sera.

A single animal to be injected with 1 ml of a $10^{-4}$ dilution of freeze-dried serum in a carrier medium of gelatin/maltose/dextran. After ALT measurements at weekly intervals for 4 weeks, if the result is negative, a second animal is started at $10^{-2}$ dilution. If the result of the first animal is positive, a second animal is injected with $10^{-6}$ dilution. Depending on the results of these two injections, a third animal will be injected with either $10^{-1}$ or $10^{-3}$ or $10^{-5}$ or $10^{-7}$ to define the infectivity to within one log over the range $10^{0}$ to $10^{-8}$. This, of course, assumes a 100% reliable infectivity model!

Total time required 5 months, total number of animals required 3.

FIG. 1

INFECTIVITY TITRATION



(iii) Inactivation titration:

A one ml vial of freeze-dried serum dilution (containing $10^4$ tamerin infective doses) to be used as a positive control in one animal, and simultaneously, 2 additional vials which have been treated by the chosen inactivation process (which may be one of: heat, γ-irradiation, adsorption, purification, detergent) and which are identical duplicates to be injected into a further two animals. ALT samples to be taken and followed for three months.

Duration of experiment 3 months, total number of animals 3.

INTERPRETATION

If the precision of the titration is ±1 log, the least infective material in the above experiment would be $10^3$ TID and if a negative result were obtained following the inoculation of the two test animals we could assume a 3 log inactivation with the chosen inactivation process, (the target for this project).

If a positive (infectious) result is obtained in the last experiment, subsequent animals will be needed for a second inactivation titration using e.g. $10^2$ TID.

Minimum period of time for all three experiments = 13 months

Minumum number of animals needed = 9.