Scottish National Blood Transfusion Service

PROTOCOL ON HEAT INACTIVATION OF LAV IN A PROTEIN STABILIZING MIXTURE

The first steps of the expertise about which we have discussed last January with Dr. Cuthberston, are summarized below:

MATERIAL

Virus = supernatant of LAV producing cell line (high titer reverse transcriptase activity 10^5 cpm/ml of supernatant)

Cells = T lymphocytes from a healthy donor stimulated for 3 days with PHA prior infection

Medium = RPMI 1640 supplemented with 10% FCS, 10% IL2 (for T cell culture), antihuman interferon and polybrene

METHODS

1) Determination of the experimental conditions:

   - for measuring reverse transcriptase in the presence of stabilizing mixture

   - for measuring LAV infectivity in the presence of the protein stabiliser

2) Heat inactivation study:

   LAV will be mixed with the stabilizing mixture (glycine - sorbitol - calcium chloride) and then heated at 60°C for various times (2 hours; 12 hours, 24 hours and 48 hours)

Controls will consist in 3 samples:

   - non heated LAV
   - LAV 2 hours at 60°C
   - LAV 24 hours at 60°C

These 7 samples will be tested for:

   a) reverse transcriptase activity in duplicate, according the method previously described (1)
b) Infectivity

3 different dilutions of each viral sample will be used to infect stimulated T lymphocytes as previously published (2). Thus, 21 T-cell cultures will be performed in duplicate and virus production will be followed twice a week during one month by measuring reverse transcriptase activity in the cell free supernatant of each culture.

REFERENCES

1) REY M.A., SPIRE B., DORMONT D., BARRE-SINOUSSE F., MONTAGNIER L. and CHERMANN J.C.
   Characterization of the RNA dependent DNA polymerase of a new human T-lymphotropic retrovirus (Lymphadenopathy Associated Virus)

2) SPIRE B., DORMONT D., BARRE-SINOUSSE F., MONTAGNIER L., CHERMANN JC.
   Inactivation of Lymphadenopathy Associated Virus by heat, gamma rays and ultraviolet light.
   LANCET; i; 1985; 188 - 189.