Dr. Perry.
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RJC/BY

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Law.

REPORT ON VISIT TO DR. R. TEDDER AND COLLEAGUES ON 18th DECEMBER 1984 - AIDS

Dr. Tedder was only available for brief periods and this report includes protracted discussions with Dr. C. H. (Sam) Cameron, Moya Briggs and Bridget (a PhD student).

The test available is: solid phase - antibody - culture lysate. Unknown serum and labelled antibody compete. The cut-off control is a known positive diluted to extinction (about 600 times, if I remember).

The test uses about 10^5 cpm and about 2% is bound in negatives (1800 counts in the run, I saw). The cut off control counts about 200 - 300 (if I remember) and positive results are clearly lower. The antibody falls during clinical AIDS but does not become negative by this test.

Both the capture and the probe antibody are simple IgG preparations from human sera. Supplies are ample. No affinity purification is needed. The reagent serum is "inactivated" by heat to remove any suspicion of gross infectivity.

The American test is: solid phase - purified antigen - unknown - enzyme labelled anti-IgG. It can be made sensitive or specific but not both.

Antigen for the American test can only be purified from cell supernate because of the purity required. Tedder's test can use cell lysate because the antigen is fixed immunologically from a crude soup. The test is intrinsically sensitive and specific.

Source of Antigen

I understood that the Gallo isolate is being grown in the permissive cell line "H9". The UK would need about 80 litres per week. As the CDC/FDA have given five firms the material to make tests, UK centres are prohibited from expanding the culture. The known firms are Abbott, Travenol, Litton and Genentech. Tedder has asked American workers to sterilise their discarded cellular material (as a short-term antigen source for the UK). They won't.

Another option is to use industrial culture facilities which

are believed to exist at CAMR Porton and which are believed to be secure enough for safe working. For some reason the DHSS have not pushed this and the preferred option seems to be to ask Wellcome to finance the work (rent-a-Porton). The British isolate (Prof. Robin Weiss at the Chester Beattie) is adapted to grow on the permissive cell line "CEM" which is thought to be available either commercially or from Montaignier. If the SNBTS really pushes the SHHD, some DHSS initiative might result in a kit, possibly to be distributed by CBLA. I do not know the detailed politics.

I believe (and told Tedder) that given cells and virus and support (scientific, not technical) from Middlesex, Scotland might go it alone.

The virus is not particularly difficult to grow (though a high level of production would be required). Tedder urgently needs an MLSO and a secretary. However, apart from improving their current service, I don't know how much this would benefit us. The culture vessel is a 500 ml Techne stirred flask similar to those used by Robin Fraser. As I said above, supplies of capture and probe antibody are secure. A test would be required for antigen, to test when a culture is ready for harvest.

After culture, the material is treated with betapropiolactone (? final concentration, ? time) before being removed from the safety cabinet for lysis by freeze-thaw. Thereafter, the reagent is treated with respect but not as highly infective.

Safety

The pressure at Middlesex derives not just from the HSE. There are members of staff who are suggesting that an inadequate level of containment is being applied. Some staff are reputed to have asked that all specimens, not just those for HTLV tests, should be handled in safety cabinets with no regard to the probable level of risk.

The cultures are processed in a level 3 containment laboratory similar to our own. However, this is in addition to their hepatitis B security laboratory. Cultures are processed in a Class 1 cabinet, except when the flasks are closed for culturing. The laboratory also contains a carbon dioxide incubator and a centrifuge (both obviously outside the cabinet).

On more general aspects, in specimen handling, they said they felt that when possible 56°C exposure for 30 minutes or 60°C for 20 minutes was likely to inactivate the virus. Safety cabinets "to some extent they appear superfluous", Dr. Cameron said, "we're not great glove people here" although most of the test processing I saw was handled by

gloved operators. Glass pippettes are not used for anything and have not been for a number of years. The nurse whose needle-stab incident was recorded in the Lancet was wearing gloves which are obviously no defence against sharp objects. Some glass test-tubes are still used but I didn't get details of how the wash-up is organised.

Specimens are only accepted for testing if they are in 1.25 ml screw-top Sarstedt plastic tubes. Such a tube may be placed inside a universal container.

On the subject of heat treatment, there was some surprise expressed that the virus in factor VIII withstands drying (though they did agree the evidence was clear). Dry heating is not thought to be a powerful option and I emphasised it was an interim solution to be followed later by full heat treatment when possible.

To test whether heat treatment is effective, it will be necessary for Tedder (T) and PFC (C) to do the following:

Culture high titre virus	(T)
Spike F VIII with it	(C)
Dry it	(C)
Show the virus survives	(T)
Measure any titre fall by quantitative assay	(T)
Spike more F VIII with enough virus to survive drying and give a significant high titre on recovery	(C)
Split the F VIII heat/no heat and dry both	(C)
Quantify the logarithmic kill attributable to the heat alone	(T)

The problems are the question of whether the PFC can carry out its end of the experiment and of when Tedder can provide a quantitative assay of viable virus. This may take 3-6 months to achieve.

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