proportion of patients if valid, durable results are to be obtained.

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Filter paper sampling of blood infected with HIV: effect of heat on antibody activity and viral infectivity

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The filter paper technique for blood sampling has not gained wide acceptance, mainly owing to incomplete standardisation. It has several advantages over venepuncture: it is less distressing for patients; samples are more easily transported; and the risk of accidental transmission of infectious agents through broken glassware and needles, etc, is reduced. We have standardised the technique.¹ Antibodies to human immunodeficiency virus (HIV) and HIV antigens have been detected in extracts of dried blood on filter papers.^{2,3} We assessed the effect of heating filter paper samples on antibody activity and infectivity of HIV.

Patients, methods, and results

We obtained venous serum samples and capillary blood collected on filter papers from 19 people attending an outpatient department for patients infected with HIV at Roslagstull Hospital for Infectious Diseases, Stockholm, and from one healthy laboratory worker. The blood samples on filter papers were dried at room temperature overnight and then either left at room temperature or heated at 56°C for one or 18 hours. A disc of 6.2 mm diameter was punched out of the filter paper and extracted in 0.5 ml phosphate saline buffer with 0.05% polysorbate 20 (Tween 20) for two hours at room temperature.¹ Serum samples were diluted 1:100 and eluates of filter paper discs used undiluted. HIV antibody activity was measured with an enzyme linked immunosorbent assay (ELISA) (Vironostika anti-HTLV-III micro-ELISA, Organon Teknika, Bostel, Holland) and western blotting performed with antigen strips (Biotech, Rockville, Maryland).

HIV infectivity was measured by using HIV obtained from freshly infected HUT-78 cells when the culture had become antigen positive in an antigen capture enzyme immunoassay (Abbott Laboratories,

North Chicago, Illinois). Discs of filter paper were soaked with 25 µl of the supernatant containing HIV and extracted in duplicate in 0.5 ml of the medium produced by Roswell Park Memorial Institute supplemented with 10% fetal calf serum. Samples were extracted immediately or after drying at room temperature or heating at 56°C for one hour. Each filter paper extract was then added to roughly 20 × 10⁶ mononuclear cells from human peripheral blood stimulated with phytohaemagglutinin, and the supernatants were assayed for HIV antigens twice weekly for six weeks. A culture was regarded as positive when the supernatant showed a rise in absorbance value on two successive occasions. Declining absorbance values were regarded as indicating a lack of replication of HIV.

Ten patients had antibodies against HIV whereas nine patients and the healthy laboratory worker did not. Activity of the antibody in the filter paper extracts was found to be equal to that shown by the diluted serum samples. The western blots obtained with filter paper extracts and serum were indistinguishable.

Heat treatment of filter papers for one hour at 56°C did not alter the optical density of extracts. Incubation for 18 hours at 56°C reduced the antibody reactivity, but in all seropositive samples it was still high. Extracts from discs soaked with supernatants from the culture containing HIV gave rising or continuously high optical densities in the assay for HIV antigens, indicating the existence of replicating virus (table). When the filter paper discs were left to dry at room temperature or heated at 56°C for one hour before extraction no replication was found.

Comment

HIV antibodies in filter paper extracts resisted heating at 56°C for one hour, whereas no replicating virus could be shown when the filter paper was left to dry at room temperature or heated at 56°C for one hour. Martin *et al* reported that HIV diluted in human serum was inactivated when heated at 56°C for 10 minutes.⁴ Resnick *et al*, however, reported that only after five hours at 56°C was the virus no longer detectable.⁵ Our results show that heating capillary blood on filter paper for one hour at 56°C efficiently inactivates HIV but not HIV antibody. More exact evaluation of this is in progress.

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Mean* optical density of culture supernatants of human mononuclear cells incubated with extracts from filter discs impregnated with HIV from freshly infected HUT-78 cells that were extracted in RPMI† medium immediately, after drying at room temperature for one hour, or after heating at 56°C for one hour. Values for discs heated at 56°C for one hour were all zero

Treatment of discs	Days of culture								
	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36	37-40
Immediate extraction	0.171	0.207	0.405	0.427	1.115	>2.000	>2.000	>2.000	>2.000
Dried at room temperature before extraction	0.134	0.091	0	0	0	0	0	0	0

*Of two values.

†Roswell Park Memorial Institute.