OXFORDSHIRE HEALTH AUTHORITY

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14th April 1993

Viricidal Activity of HB192.

The viricidal activity of HB192 was tested against Human Immunodefiency Virus type 1 (HIV 1).

Method.

1. HIV 1 (strain HTLV III/B) was propagated in CCRF-CEM cells grown in RPMI 1640 medium supplemented with 10% foetal calf serum, until virus cytopathic effect was evident.

2. 100ul of HIV culture was exposed, for 10 minutes at room temperature, to 100ul of either,
   a) HB192 as supplied,
   b) HB192 diluted 1:10 in distilled water
   c) phosphate buffered saline (PBS),
   d) PBS with subsequent heating to 60°C for 30 minutes.

   Each treatment was performed in triplicate.

3. To stop the reaction after 10 minutes, 5mls of tissue culture medium was added to each tube of reaction mixture and shaken vigorously.

4. 100ul of each of these treated cultures was taken and serial dilutions were made from 10^{-1} to 10^{-6} using uninfected CCRF-CEM culture (cell count $0.5X10^{6}$ ml^{-1}) as the diluent to give a final volume of 1 ml for each dilution.

5. These serial dilutions of treated HIV CEM cell cultures were incubated at 37°C for 21 days.

6. On days 7 and 14, a 500ul aliquot was taken from each culture and frozen at -20°C for future HIV antigen testing. Following this, the cell cultures were fed by adding 2 ml of uninfected CEM cells ($0.5X10^{6}$ ml^{-1}).

7. An Organon HIV antigen EIA was performed on each aliquot of cell culture fluid from 7, 14 and 21 days incubation according to the manufactures instructions.

Results.

	Neat HB192 treated			1:10 HB192 treated		
Day	7	14	21	7	14	21
Diln.						
-1	0.234	0.125	0.085	0.210	0.187	0.079
-2	0.110	0.091	0.067	0.080	0.078	0.065
-3	0.083	0.076	0.058	0.067	0.052	0.057
-4	0.075	0.055	0.063	0.069	0.055	0.053

	PBS+60°C treated			PBS treated		
-1	0.339	0.081	0.070	2.000	2.000	2.000
-2	0.124	0.074	0.069	2.000	2.000	2.000
-3	0.097	0.091	0.070	1.876	2.000	2.000
-4	0.098	0.091	0.071	0.987	2.000	2.000
-5	NT	NT	NT	0.255	0.981	2.000
-6	NT	NT	NT	0.088	0.079	0.070

NT: Not tested
The above readings are averages of the triplicate tests performed.

Interpretation.

This method for determining the effectiveness of a viricide for inactivation of HIV is designed to imitate the use of the product on a fresh blood spill. Both cell-free and virus infected human T-lymphocytes are present. The presence of both free virus and infected cells imitates a blood spill from an infected individual.

In this assay the effectiveness of a viricide is determined by its ability to prevent virus replication following contact of the viricide with the virus. Virus replication is detected by mixing the treated virus (viricide, PBS or PBS with heating to 60°C) with a continuous line of human T-cells which support the replication of HIV.

The best method for detecting virus replication in tissue culture is to measure the amount of HIV specific antigen (protein) present in the culture medium using a commercial enzyme immuno assay. Results from this type of assay are expressed as optical density (OD.) readings the maximum value for which OOis 2.000. If virus replication is occurring, the OD. reading will increase over time, however if replication has been prevented by inactivation by viricide, the OD. level will decrease reflecting the dilution of residual inactivated virus protein by addition of culture medium.

Exposure of HIV culture to PBS does not affect the ability of the cell-free or cell-associated virus to replicate subsequently in cell culture. This is evident from the table of results in that the amount of HIV antigen increases from day 7 to days 14 and 21 and replication occurs at a dilution of the original culture of $1:10^5$ .HIV culture heated to 60°C for 30 minutes subsequently lacks any viable virus however, reflected in a lower level of HIV antigen on day 14 compared to day 7.

Virus culture exposed to both neat and 1:10 HB192 for 10 minutes at room temperature does not subsequently contain any detectable viable HIV that replicates in tissue culture. This is demonstrated in the table of results by a low level of HIV antigen that decreases over time. Similar results are obtained with a 1:10 dilution of HB192.

Conclusion.

This report demonstrates that HB192, used neat and diluted 1:10 in water, as described above, is capable of inactivating HIV when at least an equal volume of the viricide is exposed to infected tissue culture. It must be stated however that the environment of the virus can adversely effect the activity of viricides, for example high protein concentrations can be protective. Virus present ln dried material can also be protected. Obviously the effectiveness of this viricide against HIV has only been measured under the strict conditions given above.

If there are any question you would like to ask please telephone me on Oxford 220883.

S.J.Read
Clinical Scientist

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Our ref:
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Mr. R. A. Huthwaite,
ANTECO Limited,
Stanthorpe Road,
Swadlicote, Derbyshire,
DEll 9BE.
3rd March 1992.

Dear Mr. Huthwaite,

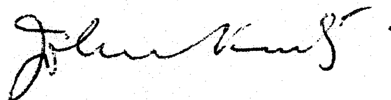
Enclosed are the results of Viricide HB192 against HBsAg. In this series, equal volumes of neat biocide (50% in reaction mixture)-columns 1,2 and 3- or of 25% biocide (12.5% in reaction mixture)- colulllllS 4,5 and 6- were mixed with HBsAg in a final concentration of 10% serum.

The results of the positive and negative controls are shown in columns 9 and 11 respectively.

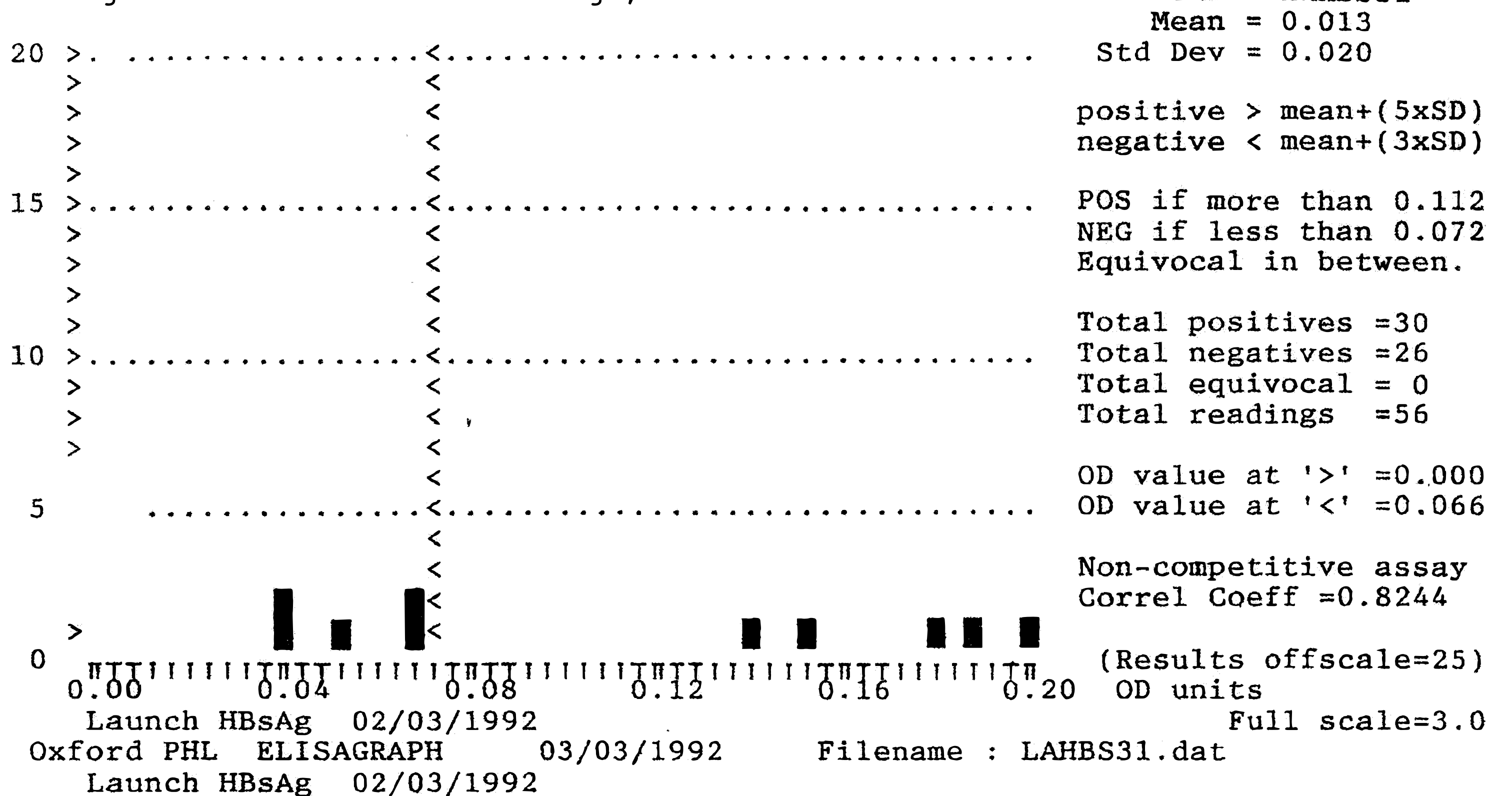
Reaction times were 10 minutes and the tests were carried out at ambient room temperature, other aspects of the test procedure were as before.

It appears that 12.5% HB192 was as effective as the 50% concent=ation of the product at the lower protein concentration {10%) used in this assay. Under these conditions, HB192 performs as well as the control biocide. These results are encouraging, please let me know if you would like further tests to be done.

Yours sincerely,



Histogram of number of readings, in 0.004 OD increments File : LAHBS31



Absorbance values

	1	2	3	4	5	6	7	8	9	10	11
A	2.386	2.392	2.366		2.009	2.024	2.112		2.058		2.766
B	1.043	0.839	0.998		1.200	1.090	1.192		0.598		2.557
C	0.457	0.414	0.356		0.177	0.185	0.150		0.199		2.396
D	0.211	0.214	0.140		0.049	0.039	0.065		0.066		1.498
E	0.006	0.006	0.009		0.013	0.006	0.007		0.012		0.514
F	0.002	0.002	0.000		0.007	0.000	0.003		0.011		0.275
G	0.004	0.003	0.002		0.004	0.002	0.000		0.000		0.039
H											

Ratios of standard deviations

	1	2	3	4	5	6	7	8	9	10	11
A	120.4	120.7	119.4		101.3	102.1	106.5		103.8		139.7
B	52.3	41.9	50.0		60.2	54.7	59.8		29.7		129.1
C	22.5	20.3	17.4		8.3	8.7	6.9		9.4		121.0
D	10.0	10.2	6.4		1.8	1.3	2.6		2.7		75.4
E	-0.4	-0.4	-0.2		-0.0	-0.4	-0.3		-0.1		25.4
F	-0.6	-0.6	-0.7		-0.3	-0.7	-0.5		-0.1		13.3
G	-0.5	-0.5	-0.6		-0.5	-0.6	-0.7		-0.7		1.3
H											

Analysis of results

	1	2	3	4	5	6	7	8	9	10	11
A	POS	POS	POS		POS	POS	POS		POS		POS
B	POS	POS	POS		POS	POS	POS		POS		POS
C	POS	POS	POS		POS	POS	POS		POS		POS
D	POS	POS	POS		-ve	-ve	-ve		-ve		POS
E	-ve	-ve	-ve		-ve	-ve	-ve		-ve		POS
F	-ve	-ve	-ve		-ve	-ve	-ve		-ve		POS
G	-ve	-ve	-ve		-ve	-ve	-ve		-ve		-ve
H											

Mean of negative group =0.013 Standard Deviation of negative group =0.020
Positive > mean+(5xSD) Positive when more than 0.112
Negative < mean+(3xSD) Negative when less than 0.072