

**Monday January 23, 1995**  
**ANTIRETROVIRAL TREATMENT OF PBLs**  
**INFECTED WITH HIV-1 JRCFS**

**Steps for testing:**

1. Prepare PHA Stimulated normal PBLs/72 hrs must stimulate in RPMI
2. Count:  $1 \times 10^7$ /ml
3. Inoculate cells/  $3 \times 10^7$  cells
4. Use JRCFS/ 10ng P24 virus  $10^6$  cells
5. JRCFS stock = 200ng/ ml
6. Inoculate 1.5 ml virus stock in  $3 \times 10^7$  cells
7. Add 1.5 ml poly
8. Incubate at  $37^\circ\text{C}$  for 2 hrs
9. Wash 2x in RPMI
10. Fresh medium at density  $1 \times 10^6$ /ml
11. Facilitate in 24 Well Plate
12. Add ANTIRETROVIRAL as follows:

**POSITIVE CONTROL**

①	②	③
	+20 ml	
⑦	⑧	⑨
	+80 ml	
⑬	⑭	⑮
	+180 ml	
⑲	⑳	㉑

**+10 ml ANTIRETROVIRAL**

④	⑤	⑥
	+40 ml	
⑩	⑪	⑫
	+160 ml	
⑰	⑱	⑲
	+200 ml	
㉒	㉓	㉔

**Report by UCLA Virology Department**  
**Notes by Amadou Diagne, PhD.**  
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**Labrador/ACLU, AIDS Virology Laboratory**

**THURSDAY JANUARY 26, 1995**  
**ANTIRETROVIRAL TREATMENT OF PBLs**  
**INFECTED WITH HIV-1 JRCFS**

**D4 Cells look good both in treated well. As in non-treated wells**

- Harvest 1 ml snips
- Same for P24
- Add fresh medium with  $5 \times 10^6$
- Incubate 'til D7

**MONDAY JANUARY 30, 1995**  
**ANTIRETROVIRAL TREATMENT OF PBLs**  
**INFECTED WITH HIV-1 JRCFS**

- Culture looks good
- Wells are all the same + Treatment with ANTIRETROVIRAL has not Affected the conditions of the cells
- Harvest all snips and terminate culture

**FRIDAY FEBRUARY 3, 1995**  
**ANTIRETROVIRAL TREATMENT OF PBLs**  
**INFECTED WITH HIV-1 JRCFS**

P24 RESULTS ng/ml

Cutoff is: 32 pf/ml

**WELL NUMBER**

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**CONTROL**

	<b>D4</b>	<b>1</b>	<b>2</b>	<b>3</b>
		<b>3.392</b>	<b>3.397</b>	<b>3.026</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>

**ANTIRETROVIRAL TREATMENT**

<b>+10 ml</b>	<b>D4</b>	<b>3.111</b>	<b>3.417</b>	<b>4.625</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>
<b>+20 ml</b>	<b>D4</b>	<b>5.332</b>	<b>4.712</b>	<b>3.671</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>
<b>+40 ml</b>	<b>D4</b>	<b>3.496</b>	<b>3.309</b>	<b>3.787</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>
<b>+80 ml</b>	<b>D4</b>	<b>3.930</b>	<b>5.512</b>	<b>4.495</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>
<b>+160 ml</b>	<b>D4</b>	<b>2.418</b>	<b>2.011</b>	<b>1.957</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>
<b>+180 ml</b>	<b>D4</b>	<b>1.698</b>	<b>1.905</b>	<b>2.180</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>
<b>+200 ml</b>	<b>D4</b>	<b>2.828</b>	<b>2.710</b>	<b>1.959</b>
	<b>D7</b>	<b>off scale</b>	<b>off scale</b>	<b>off scale</b>

**University of California, Los Angeles (1994)  
 Labrador/UCLA, AIDS Virology Laboratory**

**CVM3 TREATMENT** of Normal PHA Stimulated Peripheral Blood Lymphocytes Infected with HIV \_1 JRCFS Acquired Human Immunodeficiency Syndrome (AIDS) is a disease which targets and depletes the body's T helper cells. It is caused by the Human Immunodeficiency Virus type 1 (HIV \_1). Viral load, as measured in Peripheral Blood Mononuclear Cells (PBMCs) cultures, have been shown to correlate with early disease progression and loss of CD4 cells. Similarly suppression of viral replication by an antiretroviral agent in Vitro is a clear indication that such an agent could be a potential candidate for treatment of HIV 1 infection.

In this experiment, PHA-stimulated PBLs infected with HIV \_1 JRCSF were treated with CVM3. Supernatants were harvested on day 4 and day 7 tested for P24, to evaluate the inhibitory effect of CVM3 agent on the virus. University of California, Los Angeles (continued) we report the data here: Peripheral Blood Lymphocytes from a normal donor were stimulated in PHA - containing medium for 72 hours. Cells were washed in RPMI 1640 serum free, re-suspended in a growth medium (RPMI+20% PBS and 10 units/ml IL\_2). Cells were counted.

30 minion cells were inoculated with HIV \_1 JRCSF, at a dose of 10ng p24 virus/10 million cells. Add 15ul polybrene. Incubate at 37 degrees C, for 2 hours. Wash 2\* in serum-free RPMI.

Re-suspend in growth medium at a density of one million cell/ml  
 Distribute in a 24-well plate: 1 ml cell suspension per well.

Add CVM3 agent at different concentrations in triplicate wells. Use the first 3 wells as controls.

On day 4, culture was microscopically observed, Cells all looked healthy. There appeared to be no negative reaction due to the addition of CVM3 at any concentration: 10ul, 20ul, 40ul, 80ul, 160ul, or 200ul. Cells in control wells looked no different from those in treated wells, indicating a positive dose response. CVM3 has not affected the conditions of the cells.

On day 7, cells were microscopically examined again.  
 Similar observations were made as on day 4

**P24 RESULTS in ng/ml**

<b>CVM3 CONCENTRATE</b>	<b>WELL 1</b>	<b>WELL 2</b>	<b>WELL 3</b>
<b>10ul</b>			
<b>DAY 4</b>	<b>3.111</b>	<b>3.417</b>	<b>4.625</b>
<b>DAY 7</b>	<b>KILL</b>	<b>KILL</b>	<b>KILL</b>
<b>20ul</b>			
<b>DAY 4</b>	<b>5.332</b>	<b>4.712</b>	<b>3.671</b>
<b>DAY 7</b>	<b>KILL</b>	<b>KILL</b>	<b>KILL</b>
<b>4001</b>			
<b>DAY 4</b>	<b>3.496</b>	<b>3.309</b>	<b>3.787</b>
<b>DAY 7</b>	<b>KILL</b>	<b>KILL</b>	<b>KILL</b>