UV Effect on *Cryptosporidium parvum* Oocysts
Test III of Steri-Pen for Miles Maiden
Dated: June 29, 1999

Introduction:

This test measures the effect of UV irradiation, produced by the Steri-Pen, on the ability of *Cryptosporidium parvum* to infect mice.

Procedure:

The *C. parvum* oocysts used in this study were harvested from an infected calf on 4/10/99 and stored in 2.5% potassium dichromate at 4 C until used. The washed oocysts were added to boiled tap water (stored overnight), to make a final volume of 370 mL. in a 500 mL beaker. The temperature of the suspension was 22 C.

An initial untreated sample of 15 mL was removed from the beaker, labeled TO, and the oocyst density counted microscopically on a hemocytometer. The suspension of oocysts in water was then exposed to two successive UV irradiations, using the Steri-Pen, with gentle concomitant stirring. After 40 seconds irradiation, a 15 mL sample was removed and labeled T1. After a second exposure of 25 seconds to UV, another sample was removed and labeled T2. The second treated sample thus had been exposed for a total of 65 seconds irradiation. The oocyst density was also determined for samples T1 and T2.

In order to have greater numbers of oocysts for mouse challenges, (especially for the irradiated samples), the three samples were concentrated tenfold by centrifugation before serial dilutions were made. The number of oocysts in the irradiated samples were also counted on a hemocytometer.

Four, tenfold dilutions of the samples were prepared and 0.01 aliquots were inoculated orally into five, six-day old suckling mice, for each dilution. All mice were returned to their mothers. In the case of the irradiated samples, T1 and T2, the group receiving the greatest number of oocysts were given 0.05 mL of the 10x concentrated sample, this being the largest volume we have been able to adminster to suckling mice successfully.

Seven days after inoculation, the mice were examined for the presence, or absence of *Cryptosporidium* infection. This was done by euthanizing the mice, removing and examining their intestinal tissue microscopically under a Differential Interference Contrast (DIC) microscopic system, for the presence of oocysts. The results are presented in the accompanying table.

U.V. EFFECT ON *CRYPTOSPORIDIUM PARVUM* OOCYSTS

Mouse Infectivity **Date Infected: 6/15/99**

Samples	Dilutions*	No. *Crypto.* Oocysts Inoculated Per Mouse	# Mice Positive/5, 7 Days After inoculation	Total Mice +ve/ No Infected.
Control				
T0-0 x 10	x 10	21,400	5	5/5
T0-0	Undiluted	2,140	5	5/5
T0-1	1 : 10	214	0	0/5
T0-2	1 : 100	21	1	1/5
40 Seconds Exposure				
T1-0 x 10 x 5	x 10 x .05 mL	115,000	0	0/5
T1-0 x 10	x 10	23,000	0	0/5
T1-0	Undiluted	2,300	0	0/5
T1-1	1 : 10	230	0	0/5
40 + 25 Second Exposures				
T2-0 x 10 x 5	x 10: .05 mL	102,500	0	0/5
T2-0 x 10	x 10	20,500	0	0/5
T2-0	Undiluted	2,050	0	0/5
T2-1	1: 10	205	0	0/5

The undiluted specimens counted at 0 time and after 40 and 65 seconds, contained 21.4×10^4, 23.0×10^4, and 20.5×10^4 oocysts/ml respectively.

*** Except where indicated, the amount inoculated into each mouse was 0.01 mL.**

Conclusions:

The results of the Control (TO) series indicates that the control suspension contained infectious oocysts and that the number of oocysts required to infect a mouse was between 21 and 2,140. A statistical analysis of the data indicates that the infectious dose here was about 690 oocysts. (Reference to Statistical Table: Official Methods of Analysis of the Association of Official Analytical Chemists. 13th Edition p. 851). Other studies have shown that the expected number of oocysts to infect a mouse is 60 to 1,000 oocysts.

The results of the T1 experiment (the first UV exposure of 40 seconds) indicates that this treatment was highly effective against these oocysts. The most concentrated specimen employed here- 115,000 oocysts per mouse, failed to infect any of the mice. It is reasonable to conservatively conclude that this initial UV exposure resulted in at least a 2- log reduction in oocyst viability. The oocyst viability reduction may well have been 3 logs, or more, but we were not able to concentrate the oocysts sufficiently to test this.

As mentioned, other studies have shown that the expected infectious dose for a mouse can be as low as 60 oocysts. For example, one study (1) estimated that the dose to infect 50 percent (ID50) of neonatal Swiss-Webster mice was between 100 and 500 oocysts. Another group of investigators (2) reported the ID50 to be 60 oocysts in neonatal BALB/c mice. If Steri-Pen's performance is based on an infectious dose of 100 oocysts then Steri-Pen's treatment resulted in a minimum reduction of oocyst viability of 3.06 logs.

The second Steri-Pen exposure (T2) merely confirmed the inactivation seen after the first exposure.

These results suggest that the Steri-pen used in these experiments would probably work equally well with an even shorter UV exposure time than used here.

Researchers: Dr. Ernest Alan Meyer
Mrs. Barbara Meyer
Dr. Shivaji Ramalingam

Oregon Health Sciences University
Portland, Oregon

References

(1) Ernest, J.A. et al. 1986. Infection dynamics of *Cryptosporidium parvum* (*Apicomplexa: Cryptosporiidae*) in neonatal mice (*Mus musculus*). *Journal of Parasitology*, 72:796-798.

(2) Korich, D.G. et al. 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology*, 56:1423-1428.