Dr. Albert B. Sabin, M.D.
The Children's Hospital
Research Foundation
Elland and Bethesda
Cincinnati 29, Ohio

Dear Dr. Sabin:

In view of the lack of antigenic preservation of beta irradiated "B-virus" treated at room temperature it would seem expedient to modify the procedure and use less drastic inactivating measures. To reduce the secondary effects of peroxides and/or free radicals we can irradiate either lyophilized or frozen "B-virus" preparations. These measures have proven of some value in the past. I doubt that I could contribute much in the way of acceptable vaccine material of either frozen-irradiated or lyophilized-irradiated before the first weeks of August.

Two passages of "B-virus" in rabbit kidney cultures have yielded \(10^{8.4}\) and \(10^{8.6}\) TCID\(_{50}\)/ml when maintained in 199 medium. We will incorporate 2 percent calf serum in all future virus pools with the hope that its omission may have resulted in a half log reduction in virus titer.

It would be impossible for me to compare the sensitivity of the PFU titer with that of the titer obtained in the roller tubes. Firstly, the cells used for the PFU titer were from rabbits weighing 800 - 1200 grams; these cells were mostly fibroblast-like and seem to be less sensitive than the epithelial-like cells of younger animals. Secondly, we used an absorption period of 1 hour prior to overlay with agar. We have now come to realize that a much longer period of absorption, exceeding 4 hours, is necessary for absorption to be complete and yield titers comparable to the roller tube. We intend to critically compare these two methods sometime in the near future.

The test rabbits have now been 21 days post-challenge with 2 animals dead in the group which received \(3 \times 10^6\) irradiated material and the remaining three animals continued to appear healthy. The virus was titered on the days of challenge. We estimated 100 TCID\(_{50}\) from a surmised end point; actually each animal received 500 TCID\(_{50}\). It is possible that our method of challenge is not as sensitive as the intradermal route.
Since much work is in prospect to establish an end point of virus inactivation with frozen-irradiated (I'll use frozen material, kept at dry ice temperatures for irradiation work as we have no data on the effect of lyophilization on the virus) preparations, I can not see that I would be in a position for some weeks to test 100 ml to 1,000 ml batches critically for live virus. We will endeavor to check during each irradiation run as many of the solvent points as we can. Our space is limited and the precautions imposed prohibit rapid testing. Our first run will be made Thursday. The following protocol will be followed.

<table>
<thead>
<tr>
<th>Virus Stock Dilution</th>
<th>reps x 10^0</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^0</td>
<td>5</td>
</tr>
<tr>
<td>10^2</td>
<td>4</td>
</tr>
<tr>
<td>10^4</td>
<td>2</td>
</tr>
<tr>
<td>10^5</td>
<td>1</td>
</tr>
<tr>
<td>10^6</td>
<td>0.5</td>
</tr>
<tr>
<td>10^7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Virus titered 10^2.5/cc of 199 medium, therefore 10^0 = 10^2.5, 10^2 = 10^5.5, 10^4 = 10^7.5

This procedure will establish a series of inactivation curves based on titer, and have to date, been highly reproducible if standard conditions are maintained.

I will attempt to keep you posted as to our progress.

Sincerely yours,

THE UPJOHN COMPANY

Darwin A. Buthala, Ph.D.
Dept. of Infectious Diseases

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