MEMORANDUM

January 26, 1961

TO: Members, Enterovirus Committee

FROM: Executive Secretary, Enterovirus Committee


For your information, there is attached a list of the recommendations, motion, and resolution passed by the Enterovirus Committee at its December 8-9, 1960, meeting, which will be brought to the attention of the Virus and Cancer Panel at its forthcoming meeting February 10 and 11.

If you have any comments or suggestions concerning these recommendations, motion, or resolution, please let me know promptly so that we can change any wording that you might consider more desirable.

In addition I am also enclosing a copy of the "Guide for Use of Reference Antisera Prepared for Enteroviruses" by Dr. Wenner and the Committee.

Dr. Wenner and I are eager to get your comments and suggestions and we would appreciate receiving these at your earliest convenience. If at all possible I would ask that you send this to me by February 3 so that we will have time to prepare corrected copies for the Virus and Cancer Panel meeting February 10.

I am also enclosing for your information a table from Dr. Wenner, dated January 15, 1961, showing the current status of antiserum prepared for enteroviruses.

Attachments (3)

Marvin M. Harris, Ph.D.
Motion; Recommendations, and Resolution of Enterovirus Committee at Meeting of December 8-9, 1960

1. Enterovirus Numbering System.

RECOMMENDATION: It is recommended that the enteroviruses be brought together in a single numbering system, using as synonyms their designation as polio, Coxsackie, and ECHO viruses. As members are admitted to the group each will be given a consecutive enterovirus number. A subcommittee will undertake the task of the renumbering with polio viruses 1, 2, and 3 to be designated Enterovirus 1, 2, and 3 respectively, to be followed by the Coxsackie A's, Coxsackie B's, and ECHO viruses. Presently related types may be consolidated under a single enterovirus number.

2. Viral Typing Reagents.

RECOMMENDATION: The Committee recommends the production of two grades of viral typing reagents; one, sera for primary screening, for which high homologous titer is required, but for which cross-checking against other viruses may be deferred; and two, a high-grade reagent for viral identification for which independent confirmation of both an acceptably high homologous titer and absence of significant cross-reactivity with other viruses has been certified. Ideally, the homologous titer should be high for neutralizing, complement-fixing, and hemagglutination-inhibiting antibodies.

3. Quantity Production of Screening Sera.

RECOMMENDATION: That commercial sources for quantity production of the enterovirus reagents be explored and set up, and that the services of the Enterovirus Committee be retained for their standardization and certification. Dr. Wenner is to furnish seed antigen and perform homologous antibody titrations. Other laboratories are to confirm homologous tests.

4. Screening Antisera Production.

MOTION: Commercial on contract means be explored for the quantity production of screening diagnostic viral antisera. The Executive Secretary, Dr. Harris, is requested to explore this area and report developments to the Committee.
5. **Expansion of Present Virus Laboratory Facilities.**

RESOLUTION: The Enterovirus Committee recommends that (1) the Laboratory of Infectious Diseases of NIAID, (2) the Enterovirus Laboratory of CDC, and (3) other (non-federal) laboratories now working actively in the field of enteroviruses, be given additional support to expand their physical quarters and facilities in order to carry out the work they are doing on a broader scale. In addition, new laboratories should be recruited to work in this area.
GUIDE FOR USE OF REFERENCE ANTISERA

PREPARED FOR ENTEROVIRUSES

BY

THE COMMITTEE ON ENTEROVIRUSES

MEMBERS OF THE COMMITTEE ON ENTEROVIRUSES ARE:

Dr. Joseph L. Melnick (Chairman)
Dr. Theodore E. Boyd
Dr. Gilbert Galldorf
Dr. John F. Enders
Dr. Henry M. Gelfand
Dr. William McD. Hammon
Dr. Robert J. Huebner
Dr. Albert B. Sabin
Dr. Leon Rosen
Dr. Jerome T. Syvertson
Dr. Herbert A. Wenner

Dr. Marvin M. Harris (Executive Secretary)
GUIDE FOR USE OF REFERENCE ANTISERA PREPARED FOR ENTEROVIRUSES

At this time reference antisera have been prepared for 35 enteroviruses including 3 immunological types of poliomyelitis, 25 ECHO and 7 Coxsackie viruses. These antisera prepared in monkeys against selected prototypic viruses have been dispensed in 0.5 ml aliquots and dried by lyophilization. The dried serum is reconstituted by addition of 0.5 ml of distilled water or its equivalent.

These sera have been studied for homologous antibodies by neutralization and complement fixation methods (1,2,3,4,5). Almost all have high homologous serum dilution end points (SDE's). A few have heterologous antibodies. Major cross relationships were obtained for ECHO viruses types 1 and 8, and to a lesser extent between types 1, 8 and 12. A minor cross relationship was observed for ECHO viruses types 11 and 19; a significant one-way cross occurred with ECHO type 23 antiserum and type 22 virus. Other one-way crosses, with SDE's $\geq 1:32$ occurred with ECHO antisera types 22, 23, 24 and 25 against Group B type 4 Coxsackie virus. The Coxsackie antisera were specific within the limitations of the 7 members studied, except that the B-1 antiserum neutralized B-5 virus in dilution $= 1:128$. Antisera prepared for poliovirus were type specific; however, these antisera (6) have not been studied for heterologous antibodies against other enteroviruses. They have, however, been used extensively by other workers without any recognized difficulty in identification of polioviruses.

The Committee on Enteroviruses (1) recommended that approximately 20 units per 0.1 ml of antiserum be used for identification of unknown enteroviruses. Exceptions were noted particularly for types 5, 6, 7, 9, 10 (reovirus, type 1) and 11; these latter sera should be used at a dilution of 1:100 or
Lower, type 4 serum should be used at dilution 1:5. However, further information on the extent of intratypic variations suggest that these ranges may be too narrow. Therefore, it may now be recommended that for identification purposes high titer sera should be used initially at 1:100 dilution; low titer sera at 1:50 and very low titer sera at dilution 1:5. Under these circumstances most strains would most likely be identified, except possibly type 4.

Herbert A. Wenner, M.D.
REFERENCES

### Current Status of Antiserum Prepared for Enteroviruses

<table>
<thead>
<tr>
<th>Sera</th>
<th>Volume (mL)</th>
<th>Sera</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Coxsackie Viruses</td>
<td></td>
<td>Group B Coxsackie Viruses</td>
<td></td>
</tr>
<tr>
<td>Type 3</td>
<td>3,000</td>
<td>Type 1</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>710%</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2,100</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Completed</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1,300%</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>860%</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>700%</td>
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<tr>
<td>16</td>
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</tr>
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<td>20a</td>
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<td></td>
</tr>
<tr>
<td>20b</td>
<td>2,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>2,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B Coxsackie Viruses</td>
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<td>ECHO</td>
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</tr>
<tr>
<td>Type 6</td>
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<tr>
<td>ECHO</td>
<td></td>
<td>Type 29</td>
<td>None</td>
</tr>
<tr>
<td>Type 29</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>27</td>
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<td></td>
</tr>
<tr>
<td>28</td>
<td>2,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Commentary:** By March 15 we shall have all sera formed for Coxsackie Group A viruses which have been propagated in tissue culture. In the event of no difficulties all sera for Group A Coxsackie viruses will be formed by August 1, 1961. Standardization of these sera should be completed by February 1962.

**Antiserum Production should begin by April 1961; pools should be formed by September and standardization completed by January 1, 1962.**

*Currently being brought to 2-liter quantities.*