Epstein-Barr Virus (EBV) Intracellular Antigens: 
Factors Affecting the Patterns of Immunofluorescence

Brief Report

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With 5 Figures
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Summary

Immunofluorescent pictures of Epstein-Barr Virus (EBV) antigens were 
studied with regard to the lability of the antigens to heat and to solvents as well 
as to the modifications of the immunofluorescent patterns related to the length 
of drying time of the cell smears.

The nuclear antigen (EBNA) and the early restricted antigen (EA-R) appeared 
to be sensitive to heating 30 minutes at 56°C.

Lengthening of the drying time of the cell smears results in a progressive dis-
ersion of three antigens: viral capsid antigen (VCA), early diffuse antigen (EA-D) 
and EA-R in the cell and in some apparent loss of EBNA.

Petroleum benzine which can be used as a fixative on plastic support, allows 
the detection of all four antigens.

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The pattern of the immunofluorescent picture obtained with intracellular 
antigens allows the localization of the antigen in the host cell and, in some cases, 
allows a distinction to be made between two different antigens, e.g. the two 
components of the EBV early antigen complex: the EA-D invading both nucleus 
and cytoplasm and the EA-R condensed in cytoplasmic masses.

Moreover, the quality of the cell smears, which is a guarantee for the reli-
ability of the viral antibodies titration, can often be controlled by the pattern of 
immunofluorescence.

The method of immunofluorescence in microplates that we elaborated for the 
titration of the EBV antibodies had required some modifications of the classical 
method on glass slides, i.e. mainly: the lengthening of the drying time of the 
cell smears and the use of petroleum benzine as a fixative (3, 5).
Such modifications of the technique appeared to cause some changes in the immunofluorescence patterns that we obtained. Therefore the present investigations were carried out to determine the influence on the immunofluorescent picture of the following points: the choice of the liquid of fixation, the lengthening of the drying times and the heating of the smears.

The lymphoblastoid cell lines used as source of the antigens were: P3HR-1-K cells grown 3 days at 33°C for the VCA test (5), Raji cells from a stock culture for the EBNA test, Raji cells grown 3 days with 40 μg/ml of bromodeoxyuridin for the EA test (3).

All studies were carried out on cells cytocentrifuged on glass slides. These smears had been stored at -20°C before use.

It is to be noted that this storage did not seem to affect the antigens since no decrease of the reference serum titer could be observed on slides stored for several days when compared to the titer on slides processed immediately after cytocentrifugation. Also the patterns of immunofluorescence were similar on both types of slides.

VCA and EA-D or R were demonstrated by indirect immunofluorescence. EBNA was shown by anticomplement immunofluorescence (ACIF) (8).

Sensitivity of the antigens to heat was evaluated by titering a reference serum on the slides immediately after they had been thawed and dipped during 30 minutes in buffer at 4°C, 37°C or 56°C respectively. Results are summarized in Table 1.

VCA and EA-D were not sensitive to heating, titer of EA-R remained unchanged on slides heated at 37°C but dropped drastically on slides heated at 56°C. However the early complex had been found heat labile in some different conditions: one hour at 56°C in a moist chamber (2). EBNA showed a progressive lowering of the titer on slides heated at 37° and 56°C.

EBNA is generally considered to be thermoresistant. In fact a distinction must be made between the native antigen, demonstrated “in situ” by ACIF and the purified antigen. Recent studies had shown that the purified antigen retains its antigenicity after heating at 70° or 80°C (1, 6). However, sensitivity to heating of the native antigen in cell smears had been reported (7). Our results confirm this lability of the antigen.

In order to study the sensitivity of the antigens to solvents the cell smears were fixed by dipping them during 10 minutes in acetone, methanol or petroleum benzine at room temperature or in a mixture of methanol-acetone or of methanol-acetic acid at -20°C. Afterwards they were processed for immunofluorescence using a 1/10 dilution of a reference serum.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>30 min, 4°C</th>
<th>30 min, 37°C</th>
<th>30 min, 56°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBNA</td>
<td>1/256</td>
<td>1/64</td>
<td>1/8</td>
</tr>
<tr>
<td>EA-D</td>
<td>1/2048</td>
<td>1/2048</td>
<td>1/2048</td>
</tr>
<tr>
<td>EA-R</td>
<td>1/256</td>
<td>1/256</td>
<td>&lt;1/8</td>
</tr>
<tr>
<td>VCA</td>
<td>1/2048</td>
<td>1/2048</td>
<td>1/1024</td>
</tr>
</tbody>
</table>