INTRODUCTION
Serology is one of the keys to diagnosing EBV infection. The detection virus capsid antigen (VCA), nuclear antigen (EBNA) and early antigen (EA) antibodies makes it possible to define the status of infection (10, 14): the presence of VCA IgM and IgG in the absence of EBNA-1 IgG is considered a sign of acute infection, whereas the presence of EBNA-1 IgG and VCA IgG in the absence of VCA IgM indicates past infection (6). VCA IgM antibodies generally appear at the same time as VCA IgG antibodies, but completely disappear within a few weeks (4, 5, 9, 11, 12, 13), whereas the presence of EBNA-1 IgG and VCA IgG in the absence of VCA IgM indicates past infection (6). VCA IgM antibodies generally appear at the same time as VCA IgG antibodies; however, they completely disappear within a few weeks (4, 5, 9, 11, 12, 13), whereas the presence of EBNA-1 IgG and VCA IgG in the absence of VCA IgM indicates past infection (6).

VCA IgG antibodies persist in the body throughout the patient’s life (12). Consequently, as VCA IgG antibodies persist but EBNA-1 IgG antibodies may disappear (especially in the case of immunosuppression) (1, 2, 15), the serological pattern of EBNA-1 IgG without VCA IgG is generally considered implausible (8).

However, some immunoblotting studies have found that 2% of all past infections may be negative for VCA IgG (anti-p23 or anti-p18) antibodies (2). An “isolated EBNA-1 IgG” pattern therefore gives rise to some interpretative doubts, and it is possible to envisage aspecific EBNA-1 IgG results, or the non-production VCA IgG antibodies, or their loss over time. However, this pattern is rarely found and, in our experience, has been observed in only 1.7% of all EBNA-1 IgG-positive samples (3).

It is possible that the diversity of commercially available tests may play a major role in determining their results. For example, the antigens coated on the solid phase in ELISAs for VCA IgG may be native, synthetic or recombinant proteins that may fully or only partially represent VCA, whereas immunoblotting techniques better characterise the detected anti-EBV antibodies (11,12,13); VCA anti-p18 antibodies are produced late in the course of EBV infection, but anti-p23 antibodies are present in the case of both acute and past infections (2).

The aim of this study was to characterise the “isolated EBNA-1 IgG” pattern detected by ELISA screening tests using a quantitative VCA IgG ELISA and immunoblotting.

Presentation of the study
After screening 2422 patients in the years 2005-2006, 32 samples (1.7% of all of the samples with anti-EBNA-1 IgG) repeatedly showed the “anti-EBNA-1 IgG” pattern when tested by means of qualitative ELISA (ETI-EBNA-G, ETI-VCA-G, ETI-EBV-M reverse, DiaSorin, Saluggia, Italy). The test for the detection of VCA IgG uses the synthetic p18 protein as antigen coated to the
solid phase, and samples whose optical density (O.D.) is the same or higher than that of the “calibrator cut off” of 20 arbitrary units/ml (AU/mL) are considered positive.

Twenty-three of these samples, including three that were borderline negative for anti-VCA IgG at qualitative ELISA (-10% of the cut-off value), were tested for the presence of anti-VCA IgG antibodies by means of a quantitative ELISA (ETI-VCA-G, DiaSorin, Saluggia, Italy), in which values of ≤10 AU/mL are considered negative, values of 10-20 AU/mL are considered doubtful, and values of ≥20 AU/mL are considered positive, and were also tested for anti-EBV IgG antibodies by means of an immunoblotting assay (RecomBlot EBV IgG, MIKROGEN, Neuried, Germany) that uses recombinant antigens separated by gel-electrophoresis: p72 (EBNA-1), gp250/350 (MA), p54 and p138 (EA), and p18 and p23 (VCA).

The samples came from 23 patients referred by their general practitioners to our Microbiology Unit for serological testing: 13 males and 10 females (mean age 46 years, range 11-96) without any signs of immunodepression and who had not received any recent transfusions.

The quantitative anti-VCA IgG assay showed that four samples (17.4%) were weakly positive (21-26 AU/ml), five negative (21.7%) and 14 doubtful (60.9%). Two of the three samples that were borderline negative at qualitative ELISA were found to be positive; the third was classified as doubtful. Immunoblotting showed that all 23 samples had anti-p23 antibodies, and 13 (56.6%) weakly intense bands of anti-p18 antibodies (Table 1). Table 2 shows the presence or absence of anti-p18 antibodies in relation to the quantitative ELISA results: all of the samples that were positive at immunoblotting were positive or doubtful at quantitative ELISA.

**CONCLUSION**

Our immunoblotting findings confirm the real presence of EBNA-1 IgG and exclude a possible non-specific ELISA screening result. Moreover, the presence of anti-p23 antibodies in all of the samples (not detectable by the ELISA screening test because it only uses p18) suggests that this group of patients still showed an antibody response to the VCA. Consequently, it does not seem to be possible to have EBNA-1 IgG without VCA IgG in toto.

However, it is surprising that immunoblotting showed that over half of the samples were positive for anti-p18, whereas the quantitative VCA IgG ELISA classified them as positive or doubtful. As both the qualitative and quantitative tests use the same antigens and the same cut-off values, it is likely that constructing a curve for the quantitative test would better characterise samples that are borderline at qualitative testing. This different sensitivity may explain the discrepancy between qualitative ELISA (which uses a synthetic antigen) and immunoblotting (which uses a recombinant antigen). As the p18 immunoblotting bands were weak, these samples may have a low titre that could only be detected by immunoblotting and quantitative ELISA.

Finally, as we routinely find that 1.7% of all subjects with EBNA-1 IgG antibodies are negative at qualitative VCA IgG screening, but only about half of our samples showed anti-p18, it is possible to extrapolate that 0.96% of all EBNA-1 IgG-positive subjects do not have anti-p18 antibodies. Consequently, although anti-p18 antibodies are thought to be produced late and are therefore considered markers of past infection, they are actually not produced at all by about 1% of anti-EBNA-1 IgG-positive patients who have been affected by a past infection.

**Table 1. EBV antibodies detected by means of immunoblotting in 23 samples with an “isolated EBNA-1 IgG” pattern at ELISA screening**

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<tr>
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<tbody>
<tr>
<td>No.</td>
<td>6</td>
<td>14</td>
<td>23</td>
<td>9</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>%</td>
<td>26.1</td>
<td>60.1</td>
<td>100</td>
<td>39.1</td>
<td>100</td>
<td>56.5</td>
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**Table 2. VCA anti-p18 antibody immunoblotting results in relation to the antibody titre detected by quantitative ELISA of VCA IgG**

<table>
<thead>
<tr>
<th>Immunoblotting</th>
<th>VCA anti-p18 antibodies</th>
<th>ELISA (AU/mL)</th>
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<tbody>
<tr>
<td></td>
<td>≤10</td>
<td>&gt;10 ≤20</td>
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<tr>
<td>VCA anti-p18 -</td>
<td>5 (100%)</td>
<td>5 (35.7%)</td>
</tr>
<tr>
<td>VCA anti-p18 +</td>
<td>0 (0%)</td>
<td>9 (64.3%)</td>
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<tr>
<td>Total</td>
<td>5</td>
<td>14</td>
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* 21-26 AU/mL
REFERENCES