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Laboratory Diagnosis Of Epstein-Barr Virus As An Agent Of Acute Febrile Illness

John McMahon Jr.
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LABORATORY DIAGNOSIS
OF EPSTEIN-BARR VIRUS
AS AN AGENT OF ACUTE
FEBRILE ILLNESS

Submitted in Partial Fulfillment of the
Requirements for Graduation with Honors
to the Department of Biology at Carroll
College, Helena, Montana

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April 15, 1979
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April 15, 1979
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ABSTRACT

The use of Epstein-Barr-viral capsid antigen indirect fluorescent antibody test for infectious mononucleosis was used to study the role of the EBV in acute febrile illness of unknown etiology. Six of twenty-eight sera tested showed a four-fold rise in titer from the acute to the convalescent serum which is diagnostic evidence of EBV infection. These data suggest that EBV may play a significant role in the etiology of undiagnosed acute febrile illness and they support the thesis that testing for EB-virus is important when attempting laboratory diagnosis of acute viral disease of unknown origin.
LITERATURE REVIEW

A. INTRODUCTION

Infectious mononucleosis (IM) is an acute disease that is most prevalent in adolescents and young adults. Characteristic features of the disease include: A. clinical features such as fever, pharyngitis, lymphadenopathy, splenomegaly, and a general malaise, B. lymphocytosis with about 50% lymphocytes in the peripheral blood and C. a positive heterophil test (11, 14). This definition has now been refined and expanded by the discovery of the causal relationship between the Epstein-Barr Virus (EBV) and IM. Extensive investigations of several kinds have recently established "beyond question" the etiological role of this herpes virus in IM.

Natural primary infection with EBV generally occurs in childhood and usually does not cause symptoms in this age group (2, 14, 16). Even though these infections are apparently asymptomatic, they are always accompanied by permanent seroconversion to EBV and total immunity to IM (2, 14, 16). A unique accompaniment to EBV IM is the occurrence of "heterophil" (Paul-Bunnell-Davidsohn) antibody (Ab) in the serum of acutely ill patients; these Ab's combine specifically with antigens (Ag) found on certain mammalian erythrocytes such as horse, sheep, and bovine erythrocytes. Heterophil antibody is found in most adolescents and young adults with EBV IM but is not usually present in children.

B. HISTORY

In 1889, Emil Pfeiffer described a condition that he called glandular fever (11). Some of his patients had a short illness which
was characterized by a fever and cervical adenopathy while another group had a disease of longer duration (10 days) with sore throat and enlargement of the liver and spleen in addition to the characteristics described above. Although laboratory data were not available, these cases are thought to be the first reports of IM. In 1920 Sprunt and Evans described the first well-documented case of IM with the typical clinical features. They documented typical changes in the white blood cell (WBC) count and included a description of atypical lymphocytes. The next major contribution to IM diagnosis with the thorough study of similar atypical lymphocytes by Downey and McKinley in 1923. Their work led to a new name for these cells: "Downey Cells" (11). The next important landmark was the development of the now-famous Paul-Bunnell-Davidsohn (heterophil) test. Originally this test was performed with sheep erythrocytes (RBC's). It was later discovered that better results could be obtained with horse RBC's following beef RBC absorption and guinea-pig absorption (6).

C. ETIOLOGY

It was hypothesized that the disease may result from infection with a lymphotropic viral agent (11). Substantial support for this concept came in 1966 with the observation that humoral antibodies to EBV develop regularly during the course of IM. An indirect immunofluorescence test (IIF or IFA) to detect Ab to the virus was developed by Henle and Henle. During their studies a technician who had serviced as a seronegative control developed IM, and subsequently became seropositive. The tentative conclusion that EBV was the cause of IM was confirmed by many studies that left little reason to doubt the etiological role of the virus in the disease (2, 4, 6, 11, 14, 16, 17).
These studies can be summarized as follows: A. IM occurs only in individuals that do not have antibodies to EBV prior to infection (does not include cytomegalovirus IM) and conversely, if antibodies are present, an immunity exists for life. B. Ab's to early antigen (another Ag characteristic of EBV infections) fluctuate during the course of IM and disappear during convalescence. C. EB virus-specific IgM Ab's are present during the acute phase of IM and disappear during convalescence while IgG appears at about two weeks and will persist at a lower level for life. These can be demonstrated by means of a viral capsid antigen (VCA) test. D. The seroepidemiology of EBV and the epidemiology of IM are closely related. E. EB virus can be isolated from the throat of IM patients up to 18 months after the onset of the illness and can also be found in 15-20% of healthy persons with no history of IM (2, 14, 18). Infectious EB virus can also be detected in the saliva of 75-92% of patients with IM and has been detected as early as eight days after onset of the disease (2). F. EB virus stimulates the growth of chord blood lymphocytes in vitro and all lymphoid lines originating from the peripheral blood of acute IM patients contain the EBV genome. These results offer convincing evidence that EBV is the cause of IM.

D. EPSTEIN-BARR VIRUS

The Epstein-Barr virus is a member of the herpes virus family. Immature viral particles in the cytoplasm of infected cells measure from 75-80 uM. The mature particles, found only in the cytoplasm, have a diameter of 110-120 uM. The viral nucleoid is composed of DNA and the viral capsid surrounding it has an icosahedral symmetry with 162 subunits or capsomeres. The virus infects cells of the human lymphoreticular
system exclusively. It can be detected only in a small percentage of cells (0.3-0.5%) and some cell lines established in culture are virus-free (14).

It is thought that B-lymphocytes may be the preliminary sites of infection and only after this infection are the T-lymphocytes, which are usually the atypical lymphocytes of the disease, found (14). The virus enters the cell by means of fusion with the cell membrane. Although the capsid is usually broken and free nucleic acid migrates to the nucleus, in some cases intact nucleocapsids have been seen migrating to the nuclear membrane. The virus replicates in the areas of low chromatin density in the nucleus. The synthesis of new virions may start as early as seven hours after infection. A viral envelope is acquired by means of budding from either the Golgi membranes, the nuclear membrane itself or from the vacuoles of the cytoplasm (15).

E. EPIDEMIOLOGY AND PATHOGENESIS

The precise incidence of IM in the general population is difficult to determine. Another problem is that most "incidence studies" of IM in this and other countries have included a positive heterophil test as one of the criteria of diagnosis. It is now known that 10-25% of patients with IM in adolescent and adult age groups have a negative heterophil test (11, 12). An even higher percentage are negative in children. More clues to the incidence of IM have recently been revealed through the use of tests for Ab to EBV.

The EB virus is transmitted horizontally and affects all human population (2). As already described, natural primary infection usually takes place in childhood without typical disease manifestations. This infection is accompanied by seroconversion and lifetime persistence of the virus. This infection is first manifested as a productive infection
somewhere in the oropharynx with the liberation of small amounts of infectious virus in the buccal fluid (2, 12). There is also a second non-productive, latent infection of a small number of circulating B-lymphocytes. If natural primary infection does not occur in childhood and a susceptible individual acquires this infection as an adolescent or young adult, this event is accompanied with clinical manifestations of IM in about 50% of cases (10). This delayed natural primary infection is more frequent in middle to upper class western societies in comparison to lower socioeconomic groups. Although usually occurring in young adults, and long known to be characteristically associated with kissing in this age group, IM can also accompany primary infection in children and in adults in later life. This fact has led Rapp to state that there must be methods of transmission other than kissing (14).

The rate of occurrence of this disease seems to vary tremendously between countries (14). Studies have shown that the peak incidence of IM is in the 15 to 20 year age group. However, no definite sex or seasonal predominance has been demonstrated. There also seems to be a typical peer clustering effect. This is probably due to the mode of transmission which is most likely through the buccal fluid.

The incidence of people shedding infectious EB virus from the mouth varies in different populations and under different circumstances. In Western communities about 18-20% of healthy seropositive individuals are shedders, but this rises to 30-35% among seropositive patients on immunosuppressive therapy and to 70-90% among patients with IM (3). It has also been reported that healthy seropositives in a developing country show a 45% incidence of shedders. This is probably because endemic malaria, with its immunosuppressive effects and stimulation of
B-lymphocyte proliferation, activates latent EB virus infection. It is, of course, this infectious virus in buccal fluids that is responsible, by horizontal transmission, for natural primary infection. This is true whether the infection is silent or accompanied by symptomatic IM.

The EB virus has also been implicated in a number of other diseases. The two most important of these are Burkitt's lymphoma and a nasopharyngeal carcinoma. In Burkitt's lymphoma, severe malarial infection may constitute the selective host factor that alters the hosts' response and there is some epidemiologic evidence to support this hypothesis (13). The occurrence of Burkitt's lymphoma in childhood and predominately in non-whites may also reflect differences in predisposition (18). In the case of nasopharyngeal cancer, no ready explanation is at hand, although the high frequency of this tumor in Chinese originating in Kwangtung Province suggest a genetic factor (3).

E. CLINICAL FEATURES

The single most characteristic feature of IM is a sore throat. From 80-85% of people with IM complain of this symptom. Usually a 3-5 day period of anorexia (lack of appetite), fatigue and malaise precede this sore throat although these symptoms can be replaced by others. The four physical signs of IM that are most prevalent are: lymph node enlargement (100%), fever (98%), pharyngeal lymphatic hyperplasia (the abnormal multiplication of pharyngeal lymphocytes) (98%), and pharyngeal inflammation (85%) (1, 14). In IM, the lymph nodes are moderately tender and firm, although they are not matted down or hard and they may also vary greatly in size. Generalized lymphadenopathy and splenomegaly may also occur. The appearance of the pharyngitis in IM may vary considerably. Only a mild erythema or reddening may occur due to increased capillary flow.
At the other end of the spectrum is appearance of a greyish-white exudate which gives the uvula and the palatial arch a gelatinous appearance. A palatial enanthem, eruption of a mucous tissue, occurs in about 50% of patients with IM (1). The site of these lesions is the junction of the hard and soft palate. These lesions are sharply circumscribed, round petechiae (pinpoint, non-raised, perfectly round, purplish-red spots caused by an intradermal or submucous hemorrhage) (1). They have a diameter of 0.5 to 1.0 mm and they begin as red lesions. After 24-48 hours they turn brown or reddish brown.

In children, the diagnosis of IM is much more difficult. The disease is very difficult to diagnose because although a child may be seen with a febrile illness and a large number of atypical lymphocytes, the other symptoms are often atypical and quite variable. This problem is compounded because children frequently do not show a positive heterophil reaction. In fact, between the ages of six months and five years this heterophil reaction is almost unknown (17).

These difficulties in laboratory diagnosis have been partially alleviated by the use of EB-virus antibody titers. It is extremely likely that the majority of cases of IM in children are asymptomatic or at least subclinical. Henle and Henle have documented the EBV seroconversion in 25 children in the Cleveland Family Study. In none of these cases did an illness resembling typical IM occur in the interval between seronegative and seropositive sera (8). The possibility that these cases were subclinical has already been raised but that fact in itself would be an important finding.

In almost all cases, the sore throat subsides after two weeks. The malaise, fatigue and anorexia usually subside after three weeks while the adenopathy and splenomegaly lag slightly behind. In a few cases, the
fatigue may linger for months after the physical and laboratory findings have returned to normal. Possible complications associated with IM are numerous although they are also rare. These complications may be vascular, hematologic, hepatic or neurological in nature. A few other rare complications also may occur. These complications could prove to be fatal although IM without any complications rarely proves to be this serious.

F. LABORATORY FEATURES

Typically in the blood of the IM patient one can find a lymphocytosis of around 50% with 10-20% atypical forms. These atypical forms have been described as large with either oval, horseshoe-shaped or indented nuclei. The nuclear chromatin is dense and irregular and nucleoli are rarely found. The cytoplasm is basophilic, vacuolated, and foamy in appearance. This lymphocytosis usually is first observed between the second and the third weeks of the illness; it remains elevated for about one week.

Heterophil antibodies (HAb's) were first described by Paul Bunnell. Heterophil Ab's are defined as antibodies reactive with an antigen from another species. These HAb's agglutinate sheep, horse and ox erythrocytes. Several other types have been described such as agglutinins to serum sickness and Forssman agglutinins. The agglutinins of serum sickness are absorbed by both beef RBC and guinea-pig kidney absorption while the Forssman agglutinins are absorbed by guinea-pig absorption. The heterophil antibodies specific for IM are absorbed only by beef erythrocytes. The most important heterophil test for IM is the Paul-Bunnell-Davidsohn differential test. The basis of this test is the use of beef RBC's and guinea-pig kidney cells for absorption of sera. The beef RBC's are used to absorb serum sickness agglutinins and the guinea-pig kidney cells are
used to absorb the Forssman and serum sickness agglutinins to help to
differentiate between positive IM heterophil reaction and these other
two agglutinins.

Along with the typical heterophil reaction and the typical appearance
of atypical lymphocytes in the blood of IM patients, a new test is
proving to be very useful in the diagnosis of IM. This test is the
Epstein-Barr viral capsid antigen test (EB-VCA). This is an indirect
fluorescent antibody (IFA) test and it is a quantitative test for EB-VCA.
Since EBV has been identified as the causative agent for IM in humans,
a test for this virus is the most direct way to diagnose EBV-IM. Infection
with EBV results in the expression of several viral proteins to which
the host responds with appropriate antibodies. These antibodies are
directed against various viral antigens, the most defined being viral
capsid antigen, EB-VCA.

The indirect fluorescent antibody (IFA) test is a quantitative test
for EB-VCA which has been recommended for use in cases of IM-like disease,
and is especially useful in cases of heterophil-negative IM. The
heterophil antibody test has been shown to be less sensitive and less
specific since most children and about 10% of young adults with IM do
not convert to HAb but will be uniformly positive in the EB-VCA IFA test (9).
In heterophil positive cases of IM, the heterophil antibodies will usually
appear three days after the onset, peak in about two weeks and remain
elevated for about six weeks, after which they will begin to fall.
Antibodies to EB-VCA, however, will develop at one-two weeks in the disease,
reach a peak titer in about three-four weeks, decline to a lower level,
but then they will persist for life. This persistence can be important
because it can be used as a dependable indicator of immunity (17).
G. STATEMENT OF THESIS

The role of EB-virus in undiagnosed febrile illness remains unclear. The purpose of the present study was to investigate the importance of testing for EB-virus when attempting laboratory diagnosis of acute viral disease of undetermined etiology.
MATERIALS AND METHODS

A. SERUM SAMPLES

Samples of serum were obtained from clinical specimens submitted to the Montana State Microbiology Laboratory in Helena, MT. Pairs of sera were chosen through a series of criteria which selected for possible IM patients. Information was gathered from lab slips submitted with the specimens. These criteria were: 1. Clinical features used were the typical features of IM, namely fever, sore throat, lymphadenopathy, splenomegaly, pharyngitis and fatigue. The date of onset of the disease and the dates that the sera were taken were also considered. 2. Prior laboratory data were used to find undiagnosed pairs of sera from patients of febrile illness. Twenty-five of the twenty-eight sera tested had no previous conversion to any of the appropriate diseases tested for serologically at the state lab. These include: influenza types A & B, cytomegalovirus, rubella, rubeola, herpes simplex, hepatitis B, and toxoplasmosis. 3. Age was also used as a criterion for choosing samples, and the age group targeted was 15-25 years. This criterion was waived if it was found that other considerations such as clinical features outweighed the age criterion.

B. SOURCE OF REAGENTS

All reagents were provided commercially by Litton Bionetics Laboratory Products, Kensington, MD.

C. ANITGEN

Burkitt's lymphocytes containing EB-viral capsid antigen (EB-VCA) were used. Dried drops of an acetone-fixed suspension of these cells placed on 10-well microscope slides served as antigen preparation. At
least 10% of the Burkitt's lymphocytes prepared in this manner contain EB-VCA (18).

D. DILUTION OF PATIENTS' SERUM

Twofold serial dilution of patient sera were performed by the microtiter method. The initial dilution of 1:10 was carried to a maximum of 1:5120. Positive and negative control sera of known titer were diluted similarly. Not every dilution of every serum was tested. Dilutions were selected for testing based on the expected range of Ab titer. Patient sera were tested at the following dilutions: 1:40, 1:160, 1:320, 1:640, 1:1280. Positive control sera were tested at 1:10, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:5120 while negative control sera were tested at 1:10 and 1:20.

E. REACTION OF SERA WITH ANTIGEN

Prior to testing, each patient serum was coded by state laboratory personnel. Serum pairs were separated in this scheme. Therefore, IFA scoring was performed blind and also without knowledge of which specimens were from the acute or convalescent phase. After the testing was completed, the code was broken to evaluate the results.

Ten μl of each test dilution of each serum was added to a test slide well containing Ag. To allow patient Ab to attach to Ag, the reaction mixtures were incubated in a moist chamber for 30 minutes at 37 degrees C. Care was taken in handling to avoid mixing between the wells. After incubation, the slides were rinsed lightly with phosphate buffered saline (PBS) containing 0.01% thimerosal, pH 7.5. They were then washed twice again with PBS for five minutes, rinsed with distilled water and allowed to dry at room temperature.
F. DETECTION OF EB-VCA Ab FROM SERUM (THE IFA TEST)

Specific anti-EB-VCA Ab was revealed by attachment of fluorescein isothiocyanate (FITC)-labeled anti-human globulin Ab, as shown below:

![Diagram of EB-VCA detection](image)

**Figure-1**: Schematic of Burkitt's lymphocyte with its associated Ags & Abs

Following the Ab-Ag attachment incubation, FITC-labeled anti-human globulin Ab was allowed to react with attached patient EB-VCA Ab as follows. Ten µl of FITC-conjugate was added to each reaction well. Slides were incubated in a moist chamber as before. They were then removed and rinsed gently with PBS followed by two five-minute PBS washes. To help locate the lymphocytes during subsequent IFA examination, a counterstain was used. Slides were stained in 0.01% Evans blue in PBS for five minutes, rinsed as before with PBS and dried at room temperature.

Slides were counted with 24 X 60 mm glass coverslips and an FA mounting fluid (9:1 v/v glycerine and PBS). Care was taken to avoid and remove air bubbles which could interfere with reading the fluorescence.

Slides were read and scored using a Zeiss fluorescence microscope at 400X magnification with a 100 watt halogen illuminator with interference and excitation filters arranged to produce illumination in the 350-550 Å range. Specific fluorescence, i.e., FITC-conjugate attached to patient
Ab, appeared as brilliant apple-green fluorescence at the periphery of the lymphocytes containing EB-VCA. A number of types of non-specific fluorescence were also observed, ranging from diffuse blue-white fluorescence of the whole cell to red and yellow non-specific fluorescence.

Each dilution of each serum, including controls, was scored for intensity of fluorescence at the cell periphery by the following scheme:

- 4+ = brilliant specific fluorescence, comparable to the known positive control,
- 3+ = less brilliant but definitely positive specific fluorescence,
- 2+ = dim fluorescence of questionable specificity,
- 1+ = faint or questionable fluorescence,
- - = no fluorescence.

Endpoints were considered to be the highest dilution yielding a 3+ or greater reaction.

G. INTERPRETATION

Titers were assigned as the reciprocal of the highest serum dilution giving a positive (≥3+) result.

Increases in titer between acute and convalescent phase sera were considered significant, or diagnostic, when they were four-fold or greater.

Patient test results were considered valid when the observed positive and negative control titers fell within one two-fold dilution of the expected titer (640 and 10, respectively).
RESULTS

The results of this study are presented in figures 1 and 2.

Figure 2 shows, for each serum tested, pertinent clinical and laboratory data and the results of EB-VCA testing. All sera were selected from acute febrile illnesses; 25 of 28 pairs had shown no previous diagnostic seroconversion to various viral and mycoplasmal agents tested.

Six of the 28 pairs (21.4%) showed diagnostic rises to EB-VCA. Interestingly, three of the six also showed diagnostic rises to other viral agents including one or more of the following: cytomegalovirus, herpes simplex, rubeola, rubella, and influenza A/USSR.

Figure 3 shows the range of anti-EB-VCA titers in all sera tested. As expected from previous studies, titers in nonconverting pairs fell in the 40 to 320 range, with a mean titer of 184. Seroconversions were generally four-fold. Acute titers ranged from < 40 to 320, while convalescent converting titers ranged from 160 to 1280. For example, in the serum with the state laboratory number 9-163, the titers rose from 1:40 in the acute phase to 1:160 in the convalescent phase. This means that in the acute phase serum, the highest dilution that obtained a 3+ or greater score upon examination was the 1:40 dilution. In the convalescent phase serum, the highest dilution with a 3+ or greater score was the 1:160 dilution. Therefore, a four-fold \((4 \times 40 = 160)\) rise in titer occurred which is diagnostic evidence of EBV infection.

In all tests, control sera fell within the acceptable range of expected titers: positive controls, 320 and 640; negative controls < 10 and < 10.
Figure-2: Criteria used to choose the sera for testing by the EB-VCA IFA method and subsequent results of this testing.

This figure has been used to show the specific criteria used to pick each serum tested by the EB-VCA IFA method. All pertinent data were included when available and the results of this testing were also included.
<table>
<thead>
<tr>
<th>State lab</th>
<th>Sero-Conv. *</th>
<th>Sex/Age</th>
<th>Date of Onset</th>
<th>Clinical Features</th>
<th>Working Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-31</td>
<td>F/23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CMV</td>
</tr>
<tr>
<td>8-264</td>
<td>F/31</td>
<td>-</td>
<td>Severe cough, hoarse</td>
<td>-</td>
<td>Respiratory</td>
</tr>
<tr>
<td>8-284</td>
<td>M/49</td>
<td>02-14-78</td>
<td>-</td>
<td>-</td>
<td>CMV</td>
</tr>
<tr>
<td>8-394</td>
<td>F/25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CMV</td>
</tr>
<tr>
<td>8-411</td>
<td>M/18</td>
<td>02-14-78</td>
<td>Respiratory, gastrointestinal, fever</td>
<td>Viral Respiratory</td>
<td></td>
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<tr>
<td>8-414</td>
<td>M/20</td>
<td>03-24-78</td>
<td>Fever, enlarged lymph nodes</td>
<td>Respiratory</td>
<td></td>
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<tr>
<td>8-529</td>
<td>* M/22</td>
<td>-</td>
<td>Fever</td>
<td>-</td>
<td>CMV</td>
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<tr>
<td>8-627</td>
<td>F/04-25-78</td>
<td>Swollen glands, fever fatigue, sore throat</td>
<td>IM</td>
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<tr>
<td>9-43</td>
<td>M/38</td>
<td>-</td>
<td>Lymphadenopathy</td>
<td>CMV</td>
<td></td>
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<td>9-139</td>
<td>F/36</td>
<td>08-16-78</td>
<td>Fever, blurred vision, CNS</td>
<td>CMV Herpes</td>
<td></td>
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<tr>
<td>9-163</td>
<td>* F/25</td>
<td>-</td>
<td>Fever, lymph nodes</td>
<td>Respiratory</td>
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<tr>
<td>Laboratory Features</td>
<td>Acute Phase Serum Date/Titer</td>
<td>Convalesc. Phase Serum Date/Titer</td>
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<td>----------------------------------------------------------</td>
<td>------------------------------</td>
<td>-----------------------------------</td>
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<tr>
<td>No evidence of CMV</td>
<td>1-10-78/1:40</td>
<td>1-12-78/1:40</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>2-1-78/1:&lt; 40</td>
<td>2-15-78/1:40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversion to CMV or herpes, neg. HBs AG</td>
<td>2-17-78/1:320</td>
<td>3-1-78/1:320</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>3-8-78/1:160</td>
<td>3-28-78/1:40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seroconversion to influenza A/USSR</td>
<td>3-10-78/1:&lt; 40</td>
<td>4-6-78/1:40</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No seroconversions negative heterophil</td>
<td>3-28-78/1:160</td>
<td>4-10-78/1:&lt; 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>3-30-78/1:40</td>
<td>4-13-78/1:160</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No seroconversions negative heterophil</td>
<td>5-3-78/1:40</td>
<td>5-17-78/1:40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>7-17-78/1:320</td>
<td>8-5-78/1:320</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>8-18-78/1:40</td>
<td>8-25-78/1:40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>8-18-78/1:40</td>
<td>9-1-78/1:60</td>
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</tr>
<tr>
<td>State lab #</td>
<td>Sero-Conv.</td>
<td>Sex/ Age</td>
<td>Date of Onset</td>
<td>Clinical Features</td>
<td>Working Diagnosis</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
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</tr>
<tr>
<td>9-196</td>
<td></td>
<td>F/20</td>
<td></td>
<td>Cough, fever, chills</td>
<td>Cat Scratch CMV</td>
</tr>
<tr>
<td>9-272</td>
<td>*</td>
<td>F/15</td>
<td></td>
<td>Fever Respiratory problems</td>
<td>CMV</td>
</tr>
<tr>
<td>9-346</td>
<td></td>
<td>M/42</td>
<td>11-10-78</td>
<td>Fever</td>
<td>CMV</td>
</tr>
<tr>
<td>9-350</td>
<td></td>
<td>F/23</td>
<td></td>
<td>Fever</td>
<td>CMV herpes</td>
</tr>
<tr>
<td>9-373</td>
<td></td>
<td>M/14</td>
<td>10-1-78</td>
<td>Lymph nodes and splenomegaly</td>
<td>EBV at some time</td>
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<tr>
<td>9-384</td>
<td></td>
<td>F/21</td>
<td></td>
<td>Gastrointestinal</td>
<td>CMV</td>
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<tr>
<td>9-403</td>
<td></td>
<td>M/1</td>
<td></td>
<td>Enlarged cervical nodes and liver</td>
<td>Hepatitis CMV EBV</td>
</tr>
<tr>
<td>9-469</td>
<td></td>
<td>F/19</td>
<td></td>
<td>Hepatitis in Sep.</td>
<td>CMV Hepatitis</td>
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<tr>
<td>9-495</td>
<td>*</td>
<td>F/20</td>
<td></td>
<td>Respiratory, fever</td>
<td>Flu-like</td>
</tr>
<tr>
<td>9-544</td>
<td></td>
<td>F/3</td>
<td>1-5-79</td>
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<td>Reyes'</td>
</tr>
<tr>
<td>9-591</td>
<td>*</td>
<td>F/18</td>
<td></td>
<td>Respiratory, fever gastrointestinal</td>
<td>CMV EBV</td>
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<tr>
<td>Laboratory Features</td>
<td>Acute Phase Serum Date/ Titer</td>
<td>Convalesc. Phase Serum Date/ Titer</td>
<td></td>
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<td>--------------------------------------------</td>
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<td>-----------------------------------</td>
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<tr>
<td>No seroconversions</td>
<td>9-14-78/1:160</td>
<td>9-22-78/1:&lt;40</td>
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<td></td>
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<tr>
<td>Seroconversions to CMV and herpes</td>
<td>9-15-78/1:320</td>
<td>10-17-78/1:1280</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative heterophil no seroconversion</td>
<td>11-16-78/1:640</td>
<td>12-6-78/1:640</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>11-16-78/1:320</td>
<td>12-4-78/1:320</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>EBV-IFA-100-200 negative heterophil</td>
<td>11-15-78/1:&lt;40</td>
<td>11-27-78/1:&lt;40</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Possible recent CMV and recent herpes</td>
<td>11-15-78/1:320</td>
<td>12-5-78/1:160</td>
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<td></td>
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<tr>
<td>EBV-IFA-200-200 negative heterophil</td>
<td>12-1-78/1:160</td>
<td>12-19-78/1:160</td>
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<td></td>
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<tr>
<td>No seroconversions negative HB$_s$Ag</td>
<td>12-21-78/1:&lt;40</td>
<td>1-4-79/1:40</td>
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<tr>
<td>Rubella conversion 40-1280, rubeola conversion 64-256 pos. heter.</td>
<td>1-9-79/1:&lt;40</td>
<td>2-15-79/1:640</td>
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<td></td>
</tr>
<tr>
<td>No seroconversions</td>
<td>1-2-79/1:&lt;40</td>
<td>1-16-79/1:&lt;40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No evidence for CMV</td>
<td>1-19-79/1:40</td>
<td>2-15-79/1:160</td>
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</tr>
<tr>
<td>State Lab #</td>
<td>Sero-Conv. *</td>
<td>Sex/Age</td>
<td>Date of Onset</td>
<td>Clinical Features</td>
<td>Working Diagnosis</td>
</tr>
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<tr>
<td>9-600</td>
<td>M/7</td>
<td>1-19-79</td>
<td>Cough, fever 4 year flu</td>
<td>CMV</td>
<td>Flu-A USSR 10-160</td>
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<tr>
<td>9-646</td>
<td>* F/20</td>
<td>-</td>
<td>Respiratory, fever</td>
<td>Respiratory</td>
<td>Flu-A USSR 8-32</td>
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<tr>
<td>9-647</td>
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<td>1-8-79</td>
<td>Respiratory, fever</td>
<td>Respiratory</td>
<td>Flu-A USSR 10-80</td>
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<tr>
<td>9-654</td>
<td>M/49</td>
<td>-</td>
<td>Fever, bloody sputum</td>
<td>CMV</td>
<td>No seroconversions</td>
</tr>
<tr>
<td>9-672</td>
<td>M/62</td>
<td>-</td>
<td>-</td>
<td>EBV CMV</td>
<td>No seroconversions</td>
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<tr>
<td>9-818</td>
<td>M/20</td>
<td>-</td>
<td>Fever lymphadenopathy fatigue</td>
<td>CMV</td>
<td>No seroconversions</td>
</tr>
</tbody>
</table>
Figure-3A: Diagnostic rises in EB-VCA titer during seroconversion

This graph shows the serconversions (rises in EB-VCA titer at least four-fold) that were observed in my test sample.

A = acute phase serum of each pair
C = convalescent phase serum of each pair
----------- = titer of acute phase serum
+++++++++++ = titer of convalescent phase serum

Figure-3B: EB-VCA pair titers with no seroconversions to IM

This graph shows the typical random movement of titers in each pair. These sera pairs showed no four-fold rise in EB-VCA titer between acute and convalescent sera of each pair and therefore were not diagnostic of EBV-IM.

Legend is the same for the paired sera.

********** = the characteristic positive control titer which was obtained, $= 1:640$

= the characteristic negative control titer which was obtained,

= $\leq 10$
Figure-3A: Diagnostic rises in EB-VCA titer during seroconversion

rise in EB-VCA titer

- State laboratory numbers, A=acute & C=convalescent serum of pair

8-529
9-163
9-272
9-495
9-591
9-646
Figure-3B: EB-VCA pair titers with no seroconversions to IM

<table>
<thead>
<tr>
<th>rise in EB-VCA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 10 20 40 80 160 320 640 1280 5120</td>
</tr>
</tbody>
</table>

- State laboratory numbers, A=acute & C=convalescent serum of pair

AC 8-8-31 8-8-8-264 8-8-8-284 8-8-394 8-8-411 8-8-414 8-8-627 8-9-43 8-9-139 8-9-196 8-9-346 8-9-350
Figure-3B: (continued)

rise in EB-VCA titer

0 10 20 40 80 160 320 640 1280 5120

- State laboratory numbers, A=acute & C=convalescent serum of pair
DISCUSSION

Three conclusions can be drawn from the results. First, the EB-VCA IFA test is a very practical, reliable means of testing for specific seroconversion to the etiologic agent of IM. The precision and sensitivity of the technique make it applicable for clinical work. The fact of reliability and sensitivity is demonstrated in the results of the present study. The controls in this test easily fell into the expected range and results proved to be very reproducible. The EB-VCA IFA tests done by the Center for Disease Control generally fit the results of this study. The test was also fairly simple to do and would fit easily into a clinical laboratory time scheme.

Secondly, EB-Virus may play a significant role in the etiology of undiagnosed acute febrile illness. One fifth of the pairs tested exhibited evidence of EBV infection. This result, that 21.4% of the sera tested showed a seroconversion to EBV is very interesting and clinically relevant. All six of the twenty-eight sera that showed a seroconversion to EBV fit the general criteria for EBV-IM and probably actually reflected cases of EBV-IM.

Third, EB-VCA IFA should be performed on serum pairs under two circumstances: in undiagnosed febrile illness and in suspected IM in children under the age of ten. It has been shown repeatedly that heterophil Ab in IM rarely is seen in children (2, 3, 16, 17). The typical IM clinical symptoms are also often missing. This leads one to believe that whenever an acute febrile illness occurs that is undiagnosable by other means (heterophil or other serological evidence, especially under
the age of ten), a routine EB-VCA IFA test should be run.

An interesting finding was the seroconversion, in three pairs, to other viral agents as well as EBV. This may reflect Ab production in "malfunctioning" EBV-infected cells rather than a concomitant infection with other agents. This finding is inconsistent with the report of Tischendorf, who observed specific rises only to EB-VCA during IM (17). However, seroconversion to non-related microbial antigens has been reported during infection with other lymphotropic viruses, and seems feasible for EBV as well (17).

A number of interesting questions which may warrant further study were raised by these results.

1. Can EB-VCA testing prove useful in diagnosing heterophil negative IM in the 15-25 year age group? The present experimental design did not include this heterophil testing but recent studies have suggested that this may be a useful course of action. If the present study were repeated, heterophil testing would probably be added to the regimen (2, 5, 7). Supporting evidence derives from the fact that anti-EBV Ab is present for life after initial seroconversion in the patient. Also, even if the initial titer is high (in the 320 range) a characteristic four-fold rise can still take place.

2. Does the present dilution scheme miss some seroconversions because it misses the lower dilutions? Would more numerous and lower dilutions add precision to the test? These results seem to show that greater precision could be added with a new scheme. Three out of the twenty-eight sera tested showed a rise from < 40 to 1:40. These could be missed conversions and if this test were repeated, additional dilutions would be tested.
3. How good is the evidence that anti-EB-VCA Ab is protective against IM, considering the fact that patients with titers in the 40-320 range can still convert to IM and show typical clinical features? The answer to this question may lie in how soon the sera were drawn after onset of the disease. For the most part, date of onset was not available, and when it was available a rise in titer was observed at the expected time for typical IM (two weeks between onset and rise in titer). Therefore, the role of anti-EBV Ab in protecting against IM cannot be answered with these results. Further studies could be productive in these areas although evidence exists that a seroconversion to EBV gives permanent immunity to IM (2, 6, 12).

4. Can EB-VCA titers rise in response to infection with other agents? The work of Tischendorf suggests that this is not the case, but it seems very likely that non-EBV viral infection might lead to a reactivation of latent EB-Virus. Also, other viruses which infect lymphocytes could cause "erroneous synthesis" of anti-EB-VCA Ab.

In summary, the results support the thesis that testing for EB-Virus is important when attempting laboratory diagnosis of acute viral disease of unknown etiology.
LITERATURE CITED


