The Disease Diagnosis segment of this issue comprehends for you a disease that has many synonyms - it is simply known as infectious mononucleosis or as EBV infectious mononucleosis, Pfeiffer's disease, Filatov's disease and sometimes colloquially as the kissing disease from its oral transmission or simply as mono in North America and as glandular fever in other English-speaking countries) is an infectious, widespread viral disease caused by the Epstein–Barr virus (EBV), one type of herpes virus, to which more than 90% of adults have been exposed. Occasionally, the symptoms can recur at a later period. Most people are exposed to the virus as children, when the disease produces no noticeable or only flu-like symptoms. In developing countries, people are exposed to the virus in early childhood more often than in developed countries. As a result, the disease in its observable form is more common in developed countries. It is most common among adolescents and young adults. The disease is omnipresent the world over.

Especially in adolescents and young adults, the disease is characterized by fever, sore throat and fatigue, along with several other possible signs and symptoms. It is primarily diagnosed by observation of symptoms, but suspicion can be confirmed by several diagnostic tests.

The syndrome was described as an infectious process by Nil Filatov in 1887 and independently by Emil Pfeiffer in 1889.

It evokes a very strong reaction from the reticuloendothelial system and the body throws up what are known as Downey & McKinley cells (atypical lymphocytes) that are not of neoplastic but as bad looking and can be mistaken for neoplastic cells. Need a detailed clinico-diagnostic presentation, just flip over to the next page.

The formula HSCH₃CH₃CH(NH₂)CO₂H is that of a relatively new marker that has been linked with cardiovascular disease, high levels of which are bad enough but low levels of the same may not help. It is biosynthesized from methionine and it can be recycled into methionine or converted into cysteine with the aid of B-vitamins. Any guesses? That's right, we are talking about Homocysteine. The Interpretation section spreads an easy to understand factfile about Homocysteine.

A burning question that often raises eyebrows in Diagnostic Laboratories is - how to convey critical value reports and to whom! Troubleshooting discusses this rather tricky question for you in ample length. A critical report lying in the Laboratory while the patient is serious is actually criminal. Convey the report to the appropriate person!

Try and solve the Brain Teasers in Bouquet, honestly mark yourself. Is it all that simple as it seems?
INFECTIOUS MONONUCLEOSIS

Background: Infectious mononucleosis was first described by Sprunt and Evans in the Bulletin of the Johns Hopkins Hospital in 1920. They described the clinical characteristics of Epstein-Barr virus (EBV) infectious mononucleosis. At the time, their article was entitled "Mononuclear leukocytosis in reaction to acute infection (infectious mononucleosis)," because the causative organism, EBV, had yet to be described. Since the 1800s, infectious mononucleosis has been recognized as a clinical syndrome consisting of fever, pharyngitis, and adenopathy. The term glandular fever was first used in 1899 by German physicians and was termed Drusenfieber. The association between infectious mononucleosis and EBV was described in the late 1960s.

Frequency: EBV infectious mononucleosis is a common cause of viral pharyngitis in patients of all ages, but it is particularly frequent in young adults. In the Western world, approximately 50% of the population seroconverts before age 5 years, with much of the rest seroconverting in adolescence or young adulthood. Approximately 12% of susceptible college-aged young adults convert each year, half of whom develop acute infectious mononucleosis. In the developing world the figures are more or less the same. The disease is omnipresent the world over.

Age: Although primarily a disease of young adults, EBV infectious mononucleosis may occur from childhood to old age.

Pathophysiology: EBV is transmitted via intimate contact with body secretions, primarily oropharyngeal secretions. EBV infects the B cells in the oropharyngeal epithelium. The organism may also be shed from the uterine cervix, implicating the role of genital transmission in some cases. On rare occasion, EBV is spread via blood transfusion. Circulating B cells spread the infection throughout the entire reticular endothelial system (RES), ie, liver, spleen, and peripheral lymph nodes. EBV infection of B lymphocytes results in a humoral and cellular response to the virus. The humoral immune response directed against EBV structural proteins is the basis for the test used to diagnose EBV infectious mononucleosis. However, the T-lymphocyte response is essential in the control of EBV infection; natural killer (NK) cells and predominantly CD8+ cytotoxic T cells control proliferating B lymphocytes infected with EBV. The T-lymphocyte cellular response is critical in determining the clinical expression of EBV viral infection. A rapid and efficient T-cell response results in control of the primary EBV infection and lifelong suppression of EBV. Ineffective T-cell response may result in excessive and uncontrolled B-cell proliferation, resulting in B-lymphocyte malignancies (eg, B-cell lymphomas). The immune response to EBV infection is fever, which occurs because of cytokine release consequent to B-lymphocyte invasion by EBV. Lymphocytosis observed in the RES is caused by a proliferation of EBV-infected B lymphocytes. Pharyngitis observed in EBV infectious mononucleosis is caused by the proliferation of EBV-infected B lymphocytes in the lymphatic tissue of the oropharynx.

Mortality/Morbidity: Patients with EBV infection who present clinically with infectious mononucleosis invariably experience accompanying fatigue. Fatigue may be profound initially but usually resolves gradually in 3 months. Some patients experience prolonged fatigue and, after initial recovery, enter a state of prolonged fatigue without the features of infectious mononucleosis. Mortality and morbidity rates due to uncomplicated primary EBV infectious mononucleosis are low. The rare cases of attributed mortality are usually related to spontaneous splenic rupture. Splenic rupture may be the initial presentation of EBV mononucleosis. Most cases of EBV infectious mononucleosis are subclinical, and the only manifestation of EBV infection is a serological response to EBV surface proteins discovered with EBV serological tests. Airway obstruction and central nervous system (CNS) mononucleosis are also responsible for increased morbidity in infectious mononucleosis. Selective immunodeficiency to EBV, which occurs in persons with X-linked lymphoproliferative syndrome, may result in severe, prolonged, or even fatal infectious mononucleosis. Hepatic necrosis caused by extensive EBV proliferation in the RES of the liver is the usual cause of death in affected males. EBV is the main cause of malignant B-cell lymphomas in patients receiving organ transplants. Most instances of posttransplant lymphoproliferative disorder (PTLD) are associated with EBV. EBV in PTLD is acquired from an EBV-positive donor organ. The likelihood of PTLD is directly proportional to the degree of immunosuppressive drugs administered to the transplant patient. Depending on the intensity, rapidity, and completeness of the T-lymphocyte response, malignancy may result if EBV-induced B-lymphocyte proliferation is uncontrollable. Hodgkin disease and non-Hodgkin lymphoma (NHL) may result. Other EBV-related malignancies include oral hairy leukoplasia in patients with HIV infection. Leiomyomas and leiomyosarcomas in immunocompromised children, nasopharyngeal carcinoma, and Burkitt lymphoma are among other neoplasms caused by EBV.

History: Most patients with Epstein-Barr virus (EBV) infectious mononucleosis are asymptomatic and, therefore, have few if any symptoms. Most adults (approximately 90%) show serological evidence of previous EBV infection. The incubation period of EBV infectious mononucleosis is 1-2 months. Many patients cannot recall close contact with individuals with pharyngitis. Virtually all patients with EBV infectious mononucleosis report fatigue and prolonged malaise. A sore throat is second only to fatigue and malaise as a presenting symptom. Fever is usually present and is low grade, but chills are relatively uncommon. Arthralgias and myalgias occur but are less common than in other viral infectious diseases.

Nausea and anorexia, without vomiting, are common symptoms. Various other symptoms have been described in patients with EBV infectious mononucleosis, including cough, ocular muscle pain, chest pain, and photophobia. Importantly, patients without CNS involvement experience no cognitive difficulties. CMV infectious mononucleosis rarely involves the CNS. Myalgias, which are uncommon, are rarely (if ever) severe.

Physical Findings: Physical findings in infectious mononucleosis should be viewed in terms of frequency distribution and time course after clinical presentation. Early signs include fever, lymphadenopathy, pharyngitis, rash, and/or periorbital edema. Relative bradycardia has been described in some patients with EBV mononucleosis, but it is not a constant finding. Later physical findings include hepatomegaly, palatal petechiae, jaundice, uvarul edema, splenomegaly, and, rarely (1-2%), findings associated with splenic rupture. CNS findings associated with EBV mononucleosis are rare but usually occur later in the course of the illness. Spleenic tenderness may be present in patients with splenomegaly. Pulmonary involvement is not a feature of EBV infectious mononucleosis. The classic presentation of EBV infectious mononucleosis in children and young adults consists of the triad of fever, pharyngitis, and lymphadenopathy. Older adults and elderly patients with EBV infectious mononucleosis often have few signs and symptoms referable to the oropharynx and have little or no adenopathy. Elderly patients with EBV mononucleosis present clinically as having anicteric viral hepatitis. Predictably, jaundice develops in less than 10% of young adults with EBV infectious mononucleosis, but jaundice may occur in as many as 30% of affected elderly individuals.

The pharyngitis due to EBV infectious mononucleosis may be exudative or nonexudative. Exudative pharyngitis is commonly confused with group A streptococcal pharyngitis, which is complicated further by the fact that approximately 30% of patients with EBV infectious mononucleosis have group A streptococcal carriage of the oropharynx. The unwary physician may incorrectly conclude that a throat culture or rapid test positive for group A streptococci in a patient with infectious mononucleosis represents streptococcal pharyngitis. Nonexudative pharyngitis with or without tonsillar enlargement is common in patients with EBV infectious mononucleosis and resembles viral pharyngitis.

Patients with either exudative or nonexudative EBV infectious mononucleosis are commonly colonized by group A streptococci. Tonsillar enlargement is common, and massive tonsillar enlargement may be observed. The term kissing tonsils is used to describe extreme enlargement of both tonsils in patients with EBV infectious mononucleosis. Extreme tonsillar enlargement may result in airway obstruction. Palatal petechiae of the posterior oropharynx distinguish infectious mononucleosis from other causes of viral pharyngitis but do not distinguish it from group A streptococcal pharyngitis, in which palatal petechiae may occur. Uvarul edema is an uncommon finding in infectious mononucleosis but, if present, it is a helpful sign in distinguishing EBV infectious mononucleosis from other causes of viral pharyngitis or from group A streptococcal pharyngitis.

Early in the course of EBV infectious mononucleosis, patients may present with a maculopapular generalized rash. The rash is faint and evanescent and rapidly disappears. It is nonpruritic. This is a marked contrast to patients mistakenly diagnosed with streptococcal pharyngitis who have been administered ampicillin or amoxicillin and then develop a maculopapular rash as a drug reaction. Drug-induced rash is usually pruritic and is prolonged, in contrast to the viral rash of EBV infectious...
mononucleosis. Patients with EBV infectious mononucleosis who experience drug reactions to beta-lactams are not allergic to these medications. Administration of beta-lactams after resolution of the infection does not result in drug fevers or rashes. Splenomegaly is a late finding in EBV infectious mononucleosis. Splenic enlargement returns to normal or near normal usually within 3 weeks after the clinical presentation. In rare cases, EBV infectious mononucleosis results in various unusual clinical manifestations, including encephalitis, pancreatitis, acalculous cholecystitis, myocarditis, mesenteric adenitis, myositis, and glomerular nephritis. Neurologic syndromes due to EBV infectious mononucleosis include optic neuritis, transverse myelitis, aspecific meningitis, encephalitis, meningoencephalitis, cranial nerve (CN) palsies (particularly CN VII), and Guillain-Barré syndrome. Although EBV-induced antibodies to RBC membranes may occur; clinical anemia is uncommon with EBV infectious mononucleosis. Leukocytosis, rather than leukopenia, is the rule in infectious mononucleosis. Periorbital edema is an uncommon, and therefore fairly specific, physical finding in infectious diseases. Bilateral periorbital edema not associated with generalized edema (eg, nephrotic syndrome) suggests trichinosis, Kawasaki disease, allergic reactions, or bilateral perilobal cellulitis. Unilateral periorbital edema suggests conditions such as thyrotoxicosis, retro- orbital eye tumor, Chagas disease, insect sting, or unilateral conjunctivitis. EBV infectious mononucleosis is characterized by early and transient bilateral upper-lid edema. In contrast to the disorders mentioned above, which are either unilateral or bilateral and involve the periorbital area, with or without the eyelids, the external eye involvement of EBV infectious mononucleosis is characterized by bilateral upper-lid edema. This finding was first described by Hoagland and is referred to as Hoagland sign. Hoagland noted the association of EBV infectious mononucleosis in young military recruits with EBV infectious mononucleosis. Hoagland sign may be detected when patients look in the mirror early in the course of their illness or when the astute physician notices this early in the clinical presentation. Hoagland sign is present for only the first few days of illness and should not be sought later in the course of the infectious process.

### Differential Diagnoses of Infectious Mononucleosis

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>Epstein-Barr Virus</th>
<th>Cytomegalovirus</th>
<th>Toxoplasmosis</th>
<th>Viral Hepatitis</th>
</tr>
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<tbody>
<tr>
<td><strong>Symptoms</strong></td>
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<td></td>
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<tr>
<td>Fatigue</td>
<td>+++</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Malaise</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mild sore throat</td>
<td>+</td>
<td>+/-</td>
<td>+/−</td>
<td>+/-</td>
</tr>
<tr>
<td>Early maculopapular rash</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
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<tr>
<td><strong>Signs</strong></td>
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<tr>
<td>Early bilateral upper eyelid edema</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unilateral localized adenopathy</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bilateral posterior cervical adenopathy</td>
<td>+</td>
<td>+</td>
<td>-/−</td>
<td>+/−</td>
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<tr>
<td>Tender hepatomegaly</td>
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<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Splenomegaly</td>
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<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
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<td><strong>Laboratory abnormalities</strong></td>
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<tr>
<td>WBC count</td>
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<td>N/−</td>
<td>N</td>
<td>−</td>
</tr>
<tr>
<td>Elevated sGOT/sGPT</td>
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<td>+</td>
<td>+/-</td>
<td>+++</td>
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<td>Atypical lymphocytes (≥10%)</td>
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<td>-</td>
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<tr>
<td>Thrombocytopenia</td>
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</tr>
<tr>
<td>Elevated IgM CMV titer</td>
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<td>Elevated IgM toxoplasmosis titer</td>
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<td>-</td>
</tr>
<tr>
<td>Elevated hepatitis (eg, A, B, D, IgM) titer</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Normal**

1. Immunoglobulin M
2. IgM viral capsid antigen
3. Serum glutamic-oxaloacetic transaminase
4. Serum glutamic-pyruvic transaminase

**Causes:** The only predisposing risk factor for EBV infectious mononucleosis is close contact with an individual infected with EBV. EBV commonly persists in oropharyngeal secretions for months after clinical resolution of EBV infectious mononucleosis. Patients with congenital immunodeficiencies are predisposed to EBV-induced lymphoproliferative disorders and malignancies. Acquired immunodeficiencies due to the effects of immunosuppression (eg, PLDT) or infectious disease-induced immunosuppression (ie, HIV) may predispose to oral hairy leukoplaikia or non-Hodgkin lymphoma. Burkitt lymphoma has a distribution (ie, in Africa) that is the same as the distribution of malaria. The geographic location predisposes to Burkitt lymphoma in children.

**Laboratory Studies:** Epstein-Barr virus (EBV) infection induces specific antibodies to EBV and various unrelated non-EBV heterophile antibodies (as seen in the image below). These heterophile antibodies react to antigens from animal RBCs.

Sheep RBCs agglutinate in the presence of heterophile antibodies and are the basis for the Paul-Bunnell test. Agglutination of horse RBCs on exposure to heterophile antibodies is the basis of the Monospot test. Heterophile test antibodies are sensitive and specific for EBV heterophile antibodies, they are present in peak levels 2-6 weeks after primary EBV infection, and they may remain positive in low levels for up to a year. The latex agglutination assay using horse RBCs, is highly specific. Sensitivity is 85%, and specificity is 100%. The heterophile antibody test results may be negative early in the course of EBV infectious mononucleosis. Positivity increases during the first 6 weeks of the illness. Patients who remain heterophile negative after 6 weeks with a mononucleosis illness should be considered as having heterophile-negative infectious mononucleosis. Patients with heterophile infectious mononucleosis should be tested for EBV-specific antibodies before definitively diagnosing heterophile-negative infectious mononucleosis. Patients with heterophile- or Monospot-negative infectious mononucleosis should be tested serologically as are patients who present with a mononucleosis-like illness who are negative for heterophile antibodies. The heterophile test is less useful in children younger than 2 years, in whom the results are frequently negative. Although virtual 100% specificity exists with the agglutination test, rarely, other disorders have been reported that may produce a false-negative Monospot test result. These causes of false-negative Monospot test results include toxoplasmosis, rubella, lymphoma, and certain malignancies, particularly leukemias and/or lymphomas. Testing for EBV-specific antibodies is as follows: EBV induces a serological response to the various parts of the Epstein-Barr viral particle. IgM and IgG antibodies directed against the VCA of EBV are useful in confirming the diagnosis of EBV and in differentiating acute and/or recent infection from previous infection. EBV IgM VCA titters decrease in most patients after 3-6 months but may persist in low titer for up to 1 year. EBV IgG VCA antibodies rise later than the IgM VCA antibodies but remain elevated with variable titters for life. False-positive VCA antibody titters results may occur on the basis of cross-reactivity with other herpes viruses, eg, CMV, or with unrelated organisms, eg, Toxoplasma gondii. Other antigens indicating EBV infection are less useful diagnostically and include early antigen (EA), which is present early in EBV infectious mononucleosis. EBV nuclear antigen (EBNA) appears after 1-2 months and persists throughout life. The presence of elevated EBNA titters has the same significance as elevated IgG VCA titters. The presence of these antibodies suggests previous exposure to the antigen (past infection) and excludes EBV infection acquired in the previous year. As with heterophile antibody responses, specific EBV antibodies may not be present in children younger than 2 years. Nonspecific tests are as follows: Patients with infectious mononucleosis in the differential diagnoses should have a CBC count with differential and an evaluation of the erythrocyte sedimentation rate (ESR). The CBC count is more useful in ruling out other diagnoses that may mimic infectious mononucleosis than in providing any specific diagnostic information. Because leukocytosis is the rule in infectious mononucleosis, the presence of a normal or decreased WBC count should suggest an alternative diagnosis. Leukocytosis accompanies infectious mononucleosis, increases during the first few weeks of illness, and then gradually returns to normal. The appearance, peak, and disappearance of atypical lymphocytes follow the same time course as leukocytosis. Patients with fever, pharyngitis, and lymphadenopathy are likely to have EBV infectious mononucleosis if the relative atypical lymphocyte count is equal to or greater than 20%. Atypical lymphocytes should be differentiated from abnormal lymphocytes. Abnormal lymphocytes are associated with lymphoreticular malignancies, whereas atypical lymphocytes are...
associated with various viral and noninfectious diseases, as well as drug reactions. Atypical lymphocytes are each different in their morphology as observed on the peripheral smear, whereas abnormal lymphocytes are monotonous in their sameness, which readily permits differentiation on the peripheral smear. Because anemia is so rare with EBV infectious mononucleosis, patients with anemia should undergo workup for another cause of their anemia. Thrombocytopenia not uncommonly accompanies EBV infectious mononucleosis, but it may be present in various other viral illnesses, including in patients with heterophile-negative infectious mononucleosis. An ESR is most useful in differentiating group A streptococcal pharyngitis from EBV infectious mononucleosis. The sedimentation rate is elevated in most patients with EBV infectious mononucleosis, but it is not elevated in group A streptococcal pharyngitis. However, an elevated ESR does not differentiate EBV from the other heterophile-negative causes of infectious mononucleosis, nor does it differentiate infectious mononucleosis from malignancies. Because the liver is uniformly involved in EBV infectious mononucleosis, mild elevation of the serum transaminases is a constant finding in early EBV infectious mononucleosis. Mild increases in the serum transaminases are also a feature of the infectious agents responsible for heterophile-negative infectious mononucleosis. High elevation of the serum transaminases should suggest viral hepatitis. The serum alkaline phosphatase and gamma-glutamyl transpeptidase (GGTP) levels are not usually elevated in individuals with EBV infectious mononucleosis. Specific tests are as follows: Heterophile antibody tests: Patients with infectious mononucleosis should first be tested with a heterophile antibody test. The most commonly used is the latex agglutination assay using horse RBCs, and it is marketed under many different trade names. Enzyme-linked immunosorbent assay (ELISA) rapid diagnostic tests are also available, which are based on the detection of heterophile antibodies. Physicians should remember that heterophile antibody responses require 1–2 weeks to become positive. In a group of patients with EBV mononucleosis, the number of patients becoming positive increases to a maximum 6 weeks after the onset of the illness. If results are initially negative, a Monospot test should be ordered weekly for 6 weeks in patients with suspected EBV infectious mononucleosis. If the Monospot test remains persistently negative after 6 weeks of weekly serial testing, then a specific EBV serological test should be ordered. Before patients with an infectious mononucleosis–like syndrome are labeled as having heterophile-negative infectious mononucleosis, specific EBV serological tests should be obtained, and the results should be negative (see below). Major antibodies - Heterophile (Paul-Bunnell), EBV antigens, cold agglutinins (anti-I), smooth muscle antibodies (SMA). Minor antibodies - Rheumatoid factor (RF), antinuclear antibodies (ANA), antiphospholipid antibodies, antireticulin antibodies, antimicrosomal antibodies; anti–intermediate filaments (IMF), lymphocytotoxin, Wasserman reagin. The Monospot test has high sensitivity and specificity, eg, 85% and nearly 100%, respectively. Rarely, Monospot test results may be falsely positive, particularly in patients with CMV or rubella but also in patients with SLE and rheumatoid arthritis. Potential false-positive reactions may occur in those with HIV infection or herpes simplex virus (HSV). If a false-positive Monospot test result is suspected, then specific testing using an EBV-based antibodies serological test is indicated. A false-negative Monospot test result may occur if testing is performed too early in the course of the illness or in very young children (< 2 y) and occasionally in elderly patients. Specific EBV antibody tests: Specific EBV antibody testing is more time-consuming and expensive than the Monospot test. EBV serological tests should be obtained in patients with a mononucleosistike illness and a negative finding on the Monospot test. As with the heterophile test, the EBV antibody response may be falsely negative early in the course of the infection. False negativity may also occur in young children (< 2 y). The antibody response to specific EBV serological testing consists of measuring the antibody response to surface and core EBV viral proteins. For clinical purposes, the most useful EBV-specific antibodies are the VCA's and the EBNA. Both VCA and EBNA antibodies are usually reported as IgM or IgG antibodies. Acute infection is diagnosed in patients who have an increased EBV IgM VCA titer. Later in the course of infection, the increase in IgM VCA antibodies may be accompanied by an increase in IgG VCA antibodies and an increase in IgG EBNA antibodies. Many laboratories report EBNA titers only, which usually measure the IgG EBNA. Increased IgG VCA and/or increased IgG EBNA titers indicate past exposure to EBV, which may have been subclinical or clinical. Increased IgG VCA titers are not synonymous with chronic infectious mononucleosis, and these titers are not diagnostic of CFS. Following acute infection, the increase in IgM titers peaks after 4-8 weeks and usually remain positive for as long as 1 year. The Monospot heterophile antibodies follow the same time course as the IgM VCA titers. Rarely, cross-reactivity occurs between VCA antibodies to EBV and those to CMV or toxoplasmosis. False-positive cross-reactivity to specific EBV antibodies is extremely rare. Such patients have high elevations of IgM CAV or toxoplasmosis titers, which helps to differentiate between the primary infectious agent and the serological cross-reactivity resulting in a false-positive test result. Patients with heterophile-negative infectious mononucleosis, eg, those with persistently negative Monospot test results for 6 weeks and those with a negative EBV-specific test result, should be tested serologically for the infectious agents that cause heterophile-negative infectious mononucleosis (eg, HIV, HHV-6, toxoplasmosis, CMV, rubella, anicteric viral hepatitis). EBV Serologic Responses in EBV-Associated Diseases

<table>
<thead>
<tr>
<th>EBV Disease</th>
<th>Anti-VCA</th>
<th>Anti-EA</th>
<th>Anti-EBNA</th>
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</thead>
<tbody>
<tr>
<td>Acute EBV mononucleosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Past EBV infection</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chronic active EBV infection</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>-</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>-</td>
<td>++</td>
<td>+</td>
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</tbody>
</table>

Other tests are as follows: Patients with suspected infectious mononucleosis should not have their throats cultured for group A streptococci because the carriage rate is approximately 30% in these patients. The mere recovery of group A streptococci from the oropharynx does not signify the cause of the patient's pharyngitis; it does not differentiate colonization from infection. In such patients, a Gram stain of the oropharynx is used to differentiate patients who have pharyngitis with positive cultures for group A streptococci from those colonized with group A streptococci. Patients with EBV infectious mononucleosis or other causes of viral pharyngitis and group A streptococcal colonization have little or no white cell response on the Gram stain of the pharynx. Patients with group A streptococcal pharyngitis also have a positive finding on throat culture, but, in contrast to the patients with colonization, they show an intense polymorphonuclear cellular response with cellular debris and fibrous fragments indicating acute infection. The rapid streptococcal test cannot be used to differentiate colonization from infection any more than throat cultures. Patients with presumed CNS involvement with EBV infectious mononucleosis should undergo serological tests for other causes of viral encephalitis appropriate to the patient's exposure history. Other Tests Patients with CNS involvement may need Cland or EEG etc.

Procedures: Rarely, if ever, is a bone marrow biopsy or lymph node biopsy needed in patients with EBV infectious mononucleosis. In the diagnosis of EBV infectious mononucleosis, the assessment of lymph node enlargement can be made confidently based on specific EBV antibody testing, and surgery is almost never necessary. Patients with presumed CNS involvement with EBV infectious mononucleosis should also undergo a lumbar puncture to rule out other causes of encephalitis.

Histologic Findings: Oropharyngeal epithelium demonstrates an intense lymphoproliferative response in the cells of the oropharynx. The lymph node and spleen show lymphocytic infiltration primarily in the paracortical lymph node. Medical Care: Closely monitor patients with extreme tansieral enlargement for airway obstruction. Steroids are indicated for impending or established airway obstruction in individuals with Epstein-Barr virus (EBV) infectious mononucleosis.

Surgical Care: Surgery is necessary for spontaneous splenic rupture, which occurs in rare patients with EBV infectious mononucleosis and may be the initial manifestation of the condition.

Activity: Patients with acute EBV mononucleosis should be encouraged to rest as much as possible and to remain from active physical activity for 3 weeks.

Medication Summary: No effective antiviral therapy is available for Epstein-Barr virus (EBV) infectious mononucleosis in immunocompetent persons. Ayclovir and ganciclovir may reduce EBV shedding but are ineffective clinically. Treatment of immunocompromised patients with EBV lymphoproliferative disease is controversial. Ayclovir has not been proven to be beneficial.
Among the most important functions of a pathology or laboratory medicine service is the clear, accurate, and rapid communication of critical results (critical values) to patient care providers. Pathologists and laboratory professionals are often confronted with many obstacles in the reporting of such critical values, including establishing clinically relevant criteria for critical values, resolving difficulties in locating an ordering provider when a critical value is obtained, ensuring that the ordering provider understands the severity and implications of a critical result when he or she has questions.

**Case Scenario:** A patient with atrial fibrillation who is receiving warfarin has an afternoon cardiology appointment for routine care and anticoagulant monitoring. A basic metabolic profile and prothrombin time are ordered. The specimen is transported to the laboratory by courier, and laboratory testing is completed at 7:30 PM. All values are within normal limits except for an elevated potassium level (K) of 6.9 mEq/L (6.9 mmol/L; reference range, 3.5–5.2 mEq/L [3.5-5.2 mmol/L]) and a prothrombin time of 64.7 seconds (reference range, 11.1–13.2 seconds), corresponding to an international normalized ratio of 7.4. These results qualify as critical, and it is the laboratory's responsibility to ensure rapid communication of this critical value to the patient. The ordering physician does not respond to pages or e-mail messages, so how should the laboratory proceed?

**Critical Value Notification Procedures:** The initial step in the critical value communication process involves identification of an abnormal result by someone in the laboratory. For automated assays, the instrument, middleware, or LIS will notify the laboratory staff (usually the technical staff) when an abnormal test result is identified. The results are to be confirmed by two independent technical personnel. The automated systems in critical value reporting may serve to alert the laboratory and the appropriate personnel, but a continued shortage in the number of laboratory professionals means that fewer personnel are being directed to handle critical value notifications. Laboratories are required by numerous regulatory agencies to develop and put into practice critical value policies. Although the content of these policies varies according to institutional needs, the core components are quite similar. This article begins by describing the usual current regulatory requirements for critical value reporting. This information will be followed by a detailed analysis of the fundamental components of critical value notification. Aspects of critical value reporting that have been evaluated in the literature are emphasized, as are current technological advances that may change the way in which critical value reporting takes place.

**Establishing a Critical Values List:** Although there are many regulations specifying that laboratories must define and communicate critical values, it may seem surprising that regulations where they exist do not state which laboratory tests require critical value limits and notification. Indeed, individual clinical laboratories face unique challenges that reflect institutional organization, clinical demand, patient population, instrumentation, and staffing. Such variations have hindered the development of universal standards for critical value reporting across laboratories. The idea of a universal critical value list is appealing to many pathologists and clinicians. For example, many clinicians would likely consider a sodium (Na) level of 168 mEq/L (168 mmol/L) a “critical” value regardless of which laboratory performs the test. Indeed, the practice of assigning the laboratory director the responsibility for creating and refining the critical value list has led to similar overall inclinations. The tests between laboratories without there being a universal mandate or requirement. As an example, virtually all laboratories include Na+ on their critical value list precisely because it is important for patient care. Furthermore, not communicating a critically elevated Na+ level could have medicolegal ramifications if an adverse clinical outcome occurred. Defining (and then mandating) a universal set of thresholds for tests, however, would be a daunting task given the scarcity of outcomes-based data on critical value thresholds. Inherent variability in assay-specific reference intervals between institutions is also a complicating factor. An individual laboratory director can account for this variability by defining critical ranges consistent with his or her own assays and institutional standards. Defining critical values for tests to include in a critical value list? Moreover, how should the critical high and low thresholds be established?

Ultimately, this determination is the responsibility of the laboratory director, it should be made in communication with the clinicians who use laboratory services, as well as with a medical review board of the institution, if applicable. This task may include meeting with relevant physicians, medical and surgical section chiefs, hospital administrators, and/or nurse managers to discuss critical value policies and to determine if there are any tests that should be included (or omitted) and whether any thresholds should be adjusted according to clinical needs. Not every laboratory test should have critical values associated with it. Critical value lists are, by nature, limited to not hinder the clinical effectiveness of notification. Critical values that are too inclusive (or that have critical value thresholds that result in excessive critical value notifications) may obscure those results that are more critical. By focusing on critical values, clinicians can be exposed to critical care providers, and not to significant additional benefit to patient care. At the other extreme, lists that are too exclusive (or with thresholds that are too high or low) might not prevent adverse clinical outcomes. Both over- and under-notification of critical values by clinicians can be disastrous. A balance must be achieved. The best place to start when establishing or modifying critical value lists is by comparison with previously published lists, practice parameters, and consensus documents because these sources have been refined to benefit the clinician. A list of critical values that is based on recently published studies from CAP (Q-Probes and Q-Tracks) have compared critical value reporting across hundreds of institutions and are a valuable resource for critical value policy assessment. Although most published critical value lists do not include blood bank testing, we have included a number of transfusion medicine–related scenarios that may benefit from rapid communication and discussion with a responsible clinician.
conveyed by the nurse to the ordering physician and/or licensed caregiver. As an alternative, some hospital networks have adopted a policy of reporting all critical values generated from the outpatient setting during "off" hours to a hospital emergency department (ED) or triage center. For example, the standard operating procedure at many hospitals is to report critical results to an ED attending physician, who can then decide whether to act on these results. This approach works well for hospitals, particularly because of the extensive electronic medical record (EMR) available to all clinicians. Such a call reporting system may be of limited benefit, however, at institutions with less robust EMRs. Results would still need to be conveyed to the responsible clinician for long-term management.

**Escalation Policies:** What should a laboratory do when a technologist is not able to reach a responsible clinician with the critical value? In these circumstances, abandoning the call entirely is almost never an acceptable solution. An escalation policy or a fail-safe mechanism can be beneficial in such circumstances. An escalation policy would direct the laboratory technologist to contact a supervisor, pathology resident, and/or medical director to assist in critical value notification. This policy allows the technologist to refocus on the important task of laboratory testing, and it transfers the responsibility for notification to people who may have greater access to an inpatient or outpatient EMR and who can put the finding in a broader clinical context. In our experience, the pathology and laboratory medicine residents are usually able to contact a covering physician and convey these critical results. Verification of clinician notification should be subsequently conveyed back to the laboratory technologist (and entered into the LIS) to comply with CAP requirements. A fail-safe mechanism (or safety net) can also be used in cases in which notification continues to be unsuccessful. For example, if a "physician representing the laboratory" determines that immediate care of the patient may be required, the laboratory result and patient information might be conveyed to a physician in the ED to contact the patient directly. An approach to dealing with unreachable clinicians was recently proposed in a 2010 article. The report included algorithms for the inpatient and outpatient settings. After trying to contact the ordering provider, the algorithms include attempting to identify and contact the patient's primary care provider. If still unsuccessful, a chief of service or chief of staff would ultimately be notified. This system has an added benefit of bringing the issue of critical value reporting to the attention of a hospital or departmental administrator who might not otherwise be aware of problems associated with the process. However, as with other systems in which the ordering provider is not the physician ultimately receiving the call, this process involves clinicians who may know very little about a patient's medical history and the potential ramifications of the critical value. It is advocated for active involvement of a pathology resident, an attending pathologist, and/or a medical director in difficult-to-convey critical value calls. It has been the experience that this involvement usually opens avenues (eg, investigations via the EMR) that are not readily available to bench technologists. Such interventions can ultimately result in more rapid communication to a clinician familiar with the patient involved.

**Repeat Critical Values:** Another common problem in critical value reporting is how a laboratory should handle repeat critical values, or subsequent critical values for a given assay on the same patient (but subsequent specimen). Approximately 70% of surveyed laboratories have a policy on repeat critical values. For those that do not, one is strongly recommended because it will clarify laboratory technologist responsibility and establish consistency in performance. There are only 3 options: (1) Call only the first critical value. (2) Call each critical value. (3) Call critical values once per interval of time. As clinicians become quickly annoyed by repetitive calls for critical values, and as such calls may have diminishing value over time, some advocate using interval criteria (for example, calling once every 24 hours). Determining whether critical results meet interval criteria might add additional tasks to laboratory technologists, although LIS or middleware-based rules can be used to perform comparisons automatically. Others have suggested that interval calling is inappropriate for only select analytes. Of note, one study demonstrated that lower rates of undocumented critical value results in the medical record were associated with policies that require calling all critical results. Such an approach in high-volume laboratories, however, can be exceedingly burdensome to technologists and clinical staff, and there are minimal data to argue the clinical benefit of one approach vs the other. Critical value lists and procedures should include not just critical ranges but also the frequency of when to call for each given test. A laboratory may determine that some tests should be called with each critical value, while others (such as a markedly elevated blood urine nitrogen level) may be called using an interval approach. The laboratory policy should also clarify how to handle critical value notification after a subsequent normal result during the same interval (eg, 8 AM, critical high; 9 AM, normal; then 10 AM, critical high). In most policies, if a normal test result occurs after a critical result, a subsequent critical result is considered new and would be called again. The laboratory's policy should be clear for such scenarios.

**Critical Value Audits:** The importance of using critical value data to better understand laboratory performance assessment and to support quality improvement initiatives. The effectiveness of critical value analysis in studies of adverse events and other studies has been demonstrated. The need for a fail-safe mechanism and a clear policy for handling critical value calls is appropriate for only select analytes. Of note, one study demonstrated that lower rates of undocumented critical value results in the medical record were associated with policies that require calling all critical results. Such an approach in high-volume laboratories, however, can be exceedingly burdensome to technologists and clinical staff, and there are minimal data to argue the clinical benefit of one approach vs the other. Critical value lists and procedures should include not just critical ranges but also the frequency of when to call for each given test. A laboratory may determine that some tests should be called with each critical value, while others (such as a markedly elevated blood urine nitrogen level) may be called using an interval approach. The laboratory policy should also clarify how to handle critical value notification after a subsequent normal result during the same interval (eg, 8 AM, critical high; 9 AM, normal; then 10 AM, critical high). In most policies, if a normal test result occurs after a critical result, a subsequent critical result is considered new and would be called again. The laboratory’s policy should be clear for such scenarios.

**Brain Teasers:**

**Match the following:**

1. A. Toxoplasmosis
2. B. Lymphocytic Choriomeningitis
3. C. Psittacosis
4. D. Tularemia

**Wisdom Whispers:**

- You might get knocked down but you always keep your head up!
- There are three types of people in this world: those who make things happen, those who watch things happen and those who wonder what happened.
- Half of the troubles of this life can be traced to saying yes too quickly and not saying no soon enough.
- After A Hurricane, Comes A Rainbow.
- Tears are words the heart can't say.
- Don't judge me by the mistakes I've made, but by what I've learned from them.
- Errors of opinion may be tolerated where reason is left free to combat it.
- Marriage should be a duet - when one sings, the other claps.
- True love is quietest, except in the nascent moments of true humility.

**Brain Teasers:**

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3. C. Psittacosis
4. D. Tularemia
HOMOCYSTEINE
Structure- pictorial representation

Properties

Structure- pictorial representation

HOMOCYSTEINE is a non-protein amino acid with the formula H2NCH2CH2CO2H. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH2-) group. It is biosynthesized from methionine by the removal of its terminal C’ methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins. While detection of high levels of homocysteine has been linked to cardiovascular disease, lowering homocysteine levels may not improve outcomes.

Structure: Homocysteine exists at neutral pH values as a zwitterion.

Betaine form of (S)-homocysteine (left) and (R)-homocysteine (right)

Biosynthesis and biochemical roles: Homocysteine is not obtained from the diet. Instead, it is biosynthesized from methionine via a multi-step process. First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosyl-methionine synthetase, to give S-adenosyl methionine (SAM). SAM then transfers the methyl group to a receptor molecule, i.e., norepinephrine as an acceptor during epinephrine synthesis, DNA methyltransferase as an intermediate acceptor in the process of DNA methylation). The adenosine is then hydrolyzed to yield L-homocysteine. L-Homocysteine has two primary fates: conversion via tetrahydrofolate (THF) back into L-methionine or conversion to L-cysteine.

Biosynthesis of cysteine: Mammals biosynthesize the amino acid cysteine via homocysteine. Cystathionine β-synthase catalyzes the condensation of homocysteine and serine to give cystathionine. This reaction uses pyridoxine (vitamin B6) as a cofactor. Cystathionine β-lyase then converts this double amino acid to cysteine, ammonia, and α-ketobutyrate. Bacteria and plants rely on a different pathway to produce cysteine, relying on O-acetylserine. Two of homocysteine’s major biochemical roles. It can be synthesized from methionine and then converted back to methionine via the SAM cycle or used to create cysteine and alpha-ketobuterate.

Methionine salvage: Homocysteine can be recycled into methionine. This process uses N5-methyl tetrahydrofolate as the donor. More detail on these enzymes can be found in the article for Methionine synthesis.

Other reactions of biochemical significance: Homocysteine can cyclize to give homocysteine thiolactone, a five-membered heterocycle. Because of this “self-limited” reaction, homocysteine-containing peptides tend to cleave themselves.

Influence, proposed and verified, of homocysteine on human health

Elevated homocysteine: Deficiencies of the vitamins folic acid (B12), pyridoxine (B6), or B12 (cobalamin) can lead to high homocysteine levels. Supplementation with pyridoxine, folic acid, B12, or trimethylglycine (betaine) reduces the concentration of homocysteine in the bloodstream. Increased levels of homocysteine are linked to high concentrations of endothelial asymmetrical dimethylarginine. Recent research suggests that intense, long duration exercise raises plasma homocysteine levels, which can increase the risk of thrombosis and cardiovascular disease, which occurs more often in people with above minimal levels of homocysteine (about 6 μmol/L). These individuals require adequate dietary riboflavin in order for homocysteine levels to remain normal. Common levels in Western populations are 10 to 12 and levels of 20 μmol/L are found in populations with low B-vitamin intakes (e.g., New Delhi) or in the older elderly (e.g., Rotterdam, Framingham). Women have 10-15% less homocysteine during their reproductive decades than men, which may help explain the fact they suffer myocardial infarction (heart attacks) on average 10 to 15 years later than men.

However, this phenomenon is more readily explained by higher levels of estrogen, which exerts a cardioprotective effect.

Blood reference ranges for homocysteine

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Unit</th>
<th>Elevated limits</th>
<th>Therapeutic target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>12-19 years</td>
<td>3.3</td>
<td>7.2</td>
<td>μmol/L</td>
<td>&gt;140 μg/dL</td>
<td>&lt;6.3 μmol/L</td>
</tr>
<tr>
<td></td>
<td>&gt;60 years</td>
<td>69</td>
<td>180</td>
<td>μg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12-19 years</td>
<td>4.3</td>
<td>9.9</td>
<td>μmol/L</td>
<td>&gt;11.4 μg/dL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60 years</td>
<td>60</td>
<td>130</td>
<td>μg/dL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cardiovascular risks and related medical studies: A high level of blood serum homocysteine “Homocystinemia” is a powerful risk factor for cardiovascular disease. However, one study which attempted to decrease the risk by lowering homocysteine was not fruitful. This study was conducted on nearly 5000 Norwegian heart attack survivors who already had severe, late-stage heart disease. No study has yet been conducted in a preventive capacity on subjects who are in a relatively good state of health. However, Dr. Kilmer McCully has shown in several research studies that the development of arteriosclerosis requires elevated levels of homocysteine in the blood. Studies reported in 2006 have shown that giving vitamins (folic acid, B12, and B6) to reduce homocysteine levels may not quickly offer benefit, however a significant 25% reduction in stroke was found in the HOPE-2 study even in patients mostly with existing serious arterial decline although the overall death rate was not significantly changed by the intervention in the trial. Clearly, reducing homocysteine does not quickly repair existing structural damage of the artery architecture. However, the study is strongly supporting the biochemistry that homocysteine degrades and inhibits the formation of the three main structural components of the artery, collagen, elastin and the proteoglycans. Homocysteine permanently degrades cysteine disulfide bridges and lysine amino acid residues in proteins, gradually affecting function and structure. Simply put, homocysteine is a ‘corrosive’ of long-living proteins, i.e., collagen or elastin, or life-long proteins, i.e., fibrillin. These long-term effects are difficult to establish in clinical trials focusing on groups with existing artery decline. The main role of reducing homocysteine is possibly in ‘prevention’ but studies in patients with pre-existing conditions found no significant benefit nor damage.

Hypotheses have been offered to address the failure of homocysteine-lowering therapies to reduce cardiovascular event frequency. One suggestion is that folic acid may directly cause an increased build-up of arterial plaque, independent of its homocysteine-lowering effects. Alternatively, folic acid and vitamin B6 may cause an overall change in gene methylation levels in vascular cells, which may also promote plaque growth. Finally, altering methylation activity in cells might increase methylation of L-arginine to asymmetric dimethylarginine which can increase the risk of vascular disease. Thus alternative homocysteine-lowering therapies may yet be developed which show greater effects on development and progression of cardiovascular disease. The VITATOPS trial (results presented in May 2010 by the lead investigator, Dr Graeme J Hankey of Royal Perth Hospital, Australia at the European Stroke Conference 2010, in Barcelona, Spain) has concluded that B-vitamin supplements, within 2 years, do not seem to significantly reduce subsequent stroke, MI, or vascular death in patients with a history of recent stroke and ischemic attack, despite lowering of homocysteine levels.

Alzheimer’s disease and homocysteine: Studies demonstrate the connection between elevated levels of homocysteine (hyperhomocysteinaemia) and occurrence of Alzheimer’s disease (AD) besides other cognitive impairments. Researchers suggest that B-group-vitamin supplementation (including folate) may possibly decrease chances to develop AD.

Bone weakness and breaks: Elevated levels of homocysteine have been linked to increased fractures in elderly persons. The high level of homocysteine will auto-oxidize and react with reactive oxygen intermediates and damage endothelial cells and has a higher risk to form a thrombus. Homocysteine does not affect bone density. Instead, it appears that homocysteine affects collagen by interfering with the cross-linking between the collagen fibers and the tissues they reinforce. Whereas the HOPE-2 trial showed a reduction in stroke incidence, in those with stroke there is a high rate of hip fractures in the affected side. A trial with 2 homocysteine-lowering vitamins (folate and B12) in people with prior stroke, there was an 80% reduction in fractures, mainly hip, after 2 years. Interestingly, also here, bone density (and the number of hip falls) were increased in the women and the patients suffering from osteoporosis. However, vitamin supplements counter the deleterious effects of homocysteine on collagen. As they inefficiently absorb B12 from food, elderly persons may benefit from taking higher doses orally such as 100 mg/d (found in some multivitamins) or by intramuscular injection.
Diagnose EBV Infection with IMMUTEX

Main symptoms of Infectious mononucleosis

- Visual:
  - Photophobia

- Tonsils:
  - Reddening
  - Swelling
  - White patches

- Respiratory:
  - Cough

- Systemic:
  - Chills
  - Fever
  - Aches

- Gastric:
  - Nausea

- Lymph nodes:
  - Swelling

Stabilized suspension of specially treated horse erythrocytes.

Specific reactivity to heterophile antibodies associated with Infectious Mononucleosis.

Excludes reactivity with Frossman type of antibodies.

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Pack Size: 20 Tests