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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Hepatitis D (hepatitis delta) is a disease caused by the hepatitis D virus (HDV), a small spherical enveloped virusoid. This is one of five known hepatitis viruses: A, B, C, D, and E. HDV is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus (HBV). Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic hepatitis B or hepatitis B carrier state (superinfection).

Both superinfection and coinfection with HDV results in more severe complications compared to infection with HBV alone. These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased risk of developing liver cancer in chronic infections. In combination with hepatitis B virus, hepatitis D has the highest fatality rate of all the hepatitis infections, at 20%.

Hepatitis D virus was first reported in the mid-1977 as a nuclear antigen in patients infected with HBV who had severe liver disease. This nuclear antigen was then thought to be a hepatitis B antigen and was called the delta antigen. Subsequent experiments in chimpanzees showed that the hepatitis delta antigen (HDAg) was a structural part of a pathogen that required HBV infection to replicate. The entire genome was cloned and sequenced in 1986. It was subsequently placed in its own genus: Deltavirus.

The "**DISEASE DIAGNOSIS**" segment in this issue is all about HEPATITIS D.

As Hepatitis D is closely associated with Hepatitis B, so "**INTERPRETATION**" portion of this issue is aptly describing how to understand Hepatitis B serology in a beautiful easily assimilable tabular form.

Not to forget that the technological platform usually employed in diagnosing these disorders/ infections is ELISA. So "**TROUBLE SHOOTING**" resolves all your issues often encountered in ELISA tests.

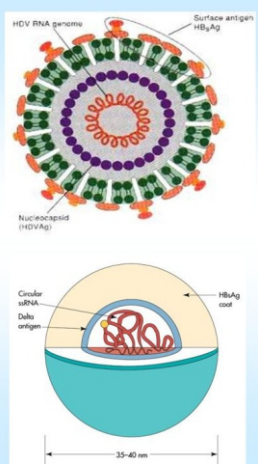
Amidst all this serious talk of Hepatitis and the related Diagnostics - can we forget a little fun and frolic. No, NEVER ! Yes, we haven't omitted "**THE BOUQUET**"

DISEASE DIAGNOSIS

HEPATITIS D

Background

Hepatitis D virus (HDV) is an RNA virus that was discovered in 1977 and is structurally unrelated to the hepatitis A (HAV), hepatitis B (HBV), and hepatitis C (HCV) viruses. HDV causes a unique infection that requires the assistance of HBV viral particles to replicate and infect hepatocytes. Its clinical course is varied and ranges from acute, self-limited infection to acute, fulminant liver failure. Chronic liver infection can lead to end-stage liver disease and associated complications (including accelerated fibrosis, liver decompensation, and hepatocellular carcinoma). There are three known genotypes of HDV. Genotype I has a worldwide distribution; genotype 2 exists in Taiwan, Japan, and northern Asia; and genotype 3 is found in South America. Simultaneous coinfection with HBV and HDV occurs in 5% of those with HBV and results in fulminant liver failure in 1% of patients. HBV-HDV superinfection is the most aggressive form of viral hepatitis. Complete clinical recovery and clearance of HBV and HDV coinfection is the most common outcome. Infection with HDV in a patient who is already positive for the hepatitis B surface antigen (HBsAg) is known as superinfection and results in fulminant liver failure in 5% of patients. Approximately 80-90% develop chronic HDV infection. These patients progress more rapidly to develop cirrhosis and may develop hepatocellular carcinoma. A study from The Netherlands suggested that HDV may hinder the control of HBV. Xiridou et al used a mathematical model for the transmission of both viruses and calculated the reproduction numbers of single HBV infections and dual HBV/HDV infections. The investigators looked at the endemic prevalence of both viruses and found that HDV modulates HBV epidemic severity and also hampers the impact on HBV interventions. Xiridou et al concluded that in endemic populations with HDV, control programs that ignore HDV presence may lead to an underestimation of the HBV epidemic and an overestimation of positive results, as control of HBV is dependent on the reproduction numbers of dual HBV/HDV infections.



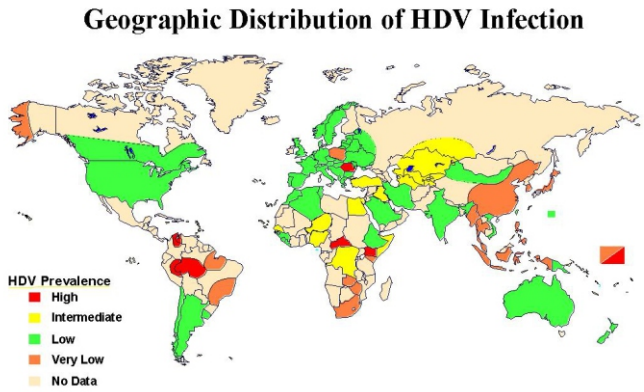
Characteristics of hepatitis D viruses

Virus	Hepatitis D
Family	Unclassified
Genus	*Deltavirus*
Virion	35 nm spherical
Envelope	Yes (HBsAg)
Genome	ssRNA
Genome size	1,7 kb
Stability	Acid-sensitive
Transmission	Parenteral
Prevalence	Low, regional
Fulminant disease	Frequent
Chronic disease	Often

Etiology

Hepatitis D virus (HDV) infection is an acute and chronic inflammatory process involving the liver. HDV is transmitted parenterally; it can replicate independently within the hepatocyte, but it requires hepatitis B surface antigen (HBsAg) for propagation. Hepatic cell death may occur due to the direct cytotoxic effect of HDV or via a host-mediated immune response. Risk factors include intravenous drug use and multiple blood transfusions. Sexual transmission is less efficient than with hepatitis B virus (HBV). Perinatal transmission is rare; no such cases have been reported in the United States.

Epidemiology



Internationally, hepatitis D virus (HDV) infection is observed more commonly among patients with a history of intravenous drug use and in persons from the Mediterranean basin. Approximately 15-20 million people are coinfected with HDV and HBV worldwide. Areas with the highest HDV prevalence include southern Italy; North Africa; the Middle East; the Amazon Basin ; and the American South Pacific islands of Samoa, Hauru, and Hiue. China, Japan, Taiwan, and Myanmar (formerly Burma) have a high prevalence of HBV infection but a low rate of HDV infection. A 2017 case-control study from Northern Spain (1983-2012) that evaluated the prevalence and epidemiology of HDV infection among those infected with HBV found an 8.2% prevalence of anti-HDV. In the analysis of patients grouped into years 1983-1997 (group A) and 1998-2012 (group B), the investigators noted the prevalence of anti-HDV fell from 9.4% in the first group to 6.1% in the second group. Moreover, independent risk factors related to the presence of anti-HDV differed between the two groups, with intravenous drug use (IVDA), blood transfusion, and high alanine aminotransferase (ALT) levels affecting the earlier group, whereas immigration, IVDA, promiscuous sexual activity, and elevated ALT levels predominantly affected the latter group. HDV infection is more common in adults than in children. However, children from underdeveloped, HDV-endemic countries are more likely to contract HDV infection through breaks in the skin, due to the presence of skin lesions.

Prognosis

The prognosis is excellent for patients with coinfection in whom treatment eradicates both viruses. The prognosis is variable for patients who are superinfected. It depends on the duration and severity of hepatitis B virus (HBV) infection, alcohol consumption, comorbid illnesses, and age. In patients who undergo liver transplantation for chronic liver disease secondary to HBV and hepatitis D virus (HDV) infections, HDV seems to suppress the replication of HBV in the transplanted liver and may help to prolong graft survival. However, fulminant hepatitis from recurrent HBV and HDV infection in the

transplanted liver has resulted in patient death or the need to retransplant. HBV-HDV superinfection significantly increases adult morbidity and mortality. In a 2017 nationwide study that evaluated risk factors associated with chronic HBV infection in France (2008-2013), investigators noted that coinfection with HDV or HCV, alcohol use disorders, diabetes mellitus, and other rare causes of chronic liver disease all increased the risk of all-cause mortality, particularly following progression of liver disease.

Complications

Complications of HDV infection may include the following:

- Liver failure
- Hepatocellular carcinoma
- Autoimmune manifestations, often including antinuclear antibodies and smooth muscle antibodies

Patient Education

Educate patients regarding modification of high-risk behaviors, including intravenous drug use and unsafe sexual practices. Promote the use of universal precautions for health care workers. Discuss with patients with chronic hepatitis D virus (HDV) and hepatitis B virus (HBV) infections that they should not donate blood, share toothbrushes or razors, or consume alcohol. Precautions should be observed regarding blood and body fluids.

HEPATITIS D CLINICAL PRESENTATION

History and Physical Examination

Hepatitis D virus (HDV) infection is clinically indistinguishable from other forms of viral hepatitis. As many as 90% of patients are asymptomatic. The incubation period is 21-45 days but may be shorter in cases of superinfection.

Signs/symptoms include the following:

- Jaundice
- Dark urine
- Abdominal pain
- Nausea with vomiting
- Confusion, bruising, and bleeding (rare)
- Pruritus

Signs/symptoms upon presentation include the following:

- Scleral icterus
- Fever
- Abdominal pain, usually right upper quadrant
- Tea-colored urine
- Encephalopathy (rare)
- Petechia with bruising (rare)

Diagnostic Considerations

The following conditions should also be considered in the differential diagnosis of hepatitis D:

- Acetaminophen poisoning
- Drug-induced hepatitis
- Fatty liver of pregnancy
- HELLP (H emolysis, E levated L iver enzymes, and L ow Platelet) syndrome in pregnant patients
- Ischemic liver injury
- Mushroom toxicity
- Bile duct strictures
- Biliary obstruction
- Conjugated hyperbilirubinemia
- Isoniazid hepatotoxicity

Differential Diagnoses

- Alcoholic Hepatitis
- Autoimmune Hepatitis
- Budd-Chiari Syndrome
- Cholangitis
- Cholecystitis
- Hepatitis A
- Hepatitis B
- Hepatitis C
- Hepatitis E
- Liver Abscess

HEPATITIS D WORKUP

Laboratory Studies

The following serum test results are present in patients with coinfection with hepatitis D virus (HDV) and hepatitis B virus (HBV):

- Results are positive for HDV antigen in 20%
- Results are positive for HDV ribonucleic acid (RNA) in 90%; reverse transcriptase polymerase chain reaction assay is currently the most sensitive assay for the detection of HDV viremia
- Results for anti-HDV immunoglobulin M (IgM) are positive initially and then are positive for anti-HDV immunoglobulin G (IgG); the finding of antigen A antibody to HDV is almost exclusively associated with chronic HDV infections
- Results for anti-HB core IgM are positive, except with superinfection, in which anti-HB core IgM is absent
- A hepatic panel may show alanine aminotransferase and aspartate aminotransferase levels greater than 500 IU/L
- For synthetic liver function markers, an international normalized ratio greater than 1.5 or a prothrombin time greater than 17 seconds may be the first evidence of fulminant liver failure

Hepatitis B surface antigen (HBsAg) is required for HDV replication but may be suppressed to undetectable levels with active HDV replication. A potentially useful, semi-automated screening assay for identifying HDV host cell requirements and antiviral targets is under investigation. It consists of a Huh-7/hNTCP cell culture–based system in a 96-well plate format, an automated microscope, and image acquisition in conjunction with CellProfiler software analysis to quantify the impact of different drugs on HDV infection (marked toxicity). Investigators found that interferons alpha-2a and beta-1a were inhibitory. When 160 human kinase inhibitors comprising all parts of the human kinome were evaluated, those that targeted the tyrosine kinase-like group had significant average anti-HDV activity, of which kenpaullone had the highest selective index.

Imaging Studies

Right upper quadrant ultrasonography helps in the evaluation of biliary obstruction and hepatocellular carcinoma. Perform cholescintigraphy (hydroxy iminodiacetic acid) to exclude acute cholecystitis, if clinically indicated. Perform computed tomography (CT) scanning or magnetic

resonance imaging (MRI) if hepatocellular carcinoma is suspected. (An alpha-fetoprotein [AFP] level greater than 250 ng/mL is highly suggestive of hepatocellular carcinoma [HCC].)

Histologic Findings

Results from liver biopsy in patients with acute disease are consistent with acute hepatitis, and, generally, a biopsy is not indicated. Consider liver biopsy if the serologic diagnosis of hepatitis is inconclusive. In patients with chronic liver disease, liver biopsy is indicated to evaluate for the presence of fibrosis and cirrhosis. HDV antigen immunohistochemical analysis of liver tissue is the criterion standard for establishing a diagnosis of persistent HDV infection. Histologic features are very similar to those observed in patients with HBV infection. Acidophilic bodies and degeneration of hepatocytes with acidophilic cytoplasm are present. The few inflammatory cells (lymphocytes) likely represent the direct cytotoxicity of HDV. Results of immunohistochemical staining for HDV antigen are positive. With superinfection, staining often reveals that HBsAg is suppressed.

HEPATITIS D TREATMENT & MANAGEMENT

Approach Considerations

Treatment for infection with hepatitis D virus (HDV) consists primarily of supportive measures (in part owing to the fact that HDV is very host dependent and absent of potentially drugable enzyme in its genome). Observe synthetic liver function markers and mental status closely. Deterioration of either should prompt early consultation with hospital personnel capable of performing liver transplantation. Liver transplantation is indicated in patients with fulminant liver failure. Patients with evidence of decompensated liver disease or fulminant liver failure should be immediately transferred to a center capable of performing a liver transplantation. No pharmacologic treatment for HDV has been approved. Peginterferon alfa-2a (PEG-IFNa2a) and nucelos(t)ide analogues have been used to manage chronic HBV infection, but only PEG-IFN has shown anti-HDV activity. However, a study of the efficacy of PEG-IFNa2a found that treatment with or without adefovir over 48 weeks resulted in sustained HDV RNA clearance in approximately one fourth of patients. In another study, PEG-IFN achieved sustained viral response (SVR) and remission in only 29.4% of patients. Thus, PEG-IFNa has low rates of SVR and clinical improvement. The efficacy rate of interferon-based therapy does not exceed 30%, with frequent termination of therapy owing to serious side effects, and the relapse rate is very high. Potential new therapies remain under investigation, including prenylation inhibitors (against HDV only), as well as viral entry inhibitors and HBsAg-release inhibitors (against HDV and hepatitis B virus [HBV] coinfection). No vaccine is available for HDV, but the HBV vaccination is effective against HDV.

Consultations

Early notification of a hepatologist or gastroenterologist is warranted.

Diet

Diet need not be restricted. If enteral intake is poor, intravenous fluids can be administered. Total parental nutrition is seldom needed.

Prevention

Cost-effective, optimal strategies to reduce the prevalence of hepatitis B virus (HBV) in moderately hepatitis D virus (HDV)-endemic regions include the implementation of all four of the following interventions :

- Testing, with HBV adult vaccination (diagnosis)
- Diagnosis, with anti-HBV therapy (mono-infections)
- Diagnosis, with combined anti–HBV-HDV therapy (dual infections)
- Creation and utilization of effective awareness programs.

Long-Term Monitoring

Follow-up is recommended for at least 6 months to determine if chronic hepatitis B virus (HBV) and hepatitis D virus (HDV) infection develop. Perform a liver biopsy to stage liver disease prior to beginning interferon alfa therapy. Treatment with interferon can be continued after the 1-year period if well tolerated and efficacy is demonstrated. Monitoring HDV RNA and hepatitis B surface antigen (HBsAg) levels may help in guiding therapy.

Medication Summary

Antiviral therapy with interferon alfa can be considered in patients with chronic hepatitis D virus (HDV) infection. The treatment course is usually at least 1 year. Treating children with interferon alfa seems to be safe but is relatively ineffective. Treatment is not needed for patients with coinfection, given the high spontaneous clearance rates. Lamivudine, ribavirin, and corticosteroids have not been effective in treatment.

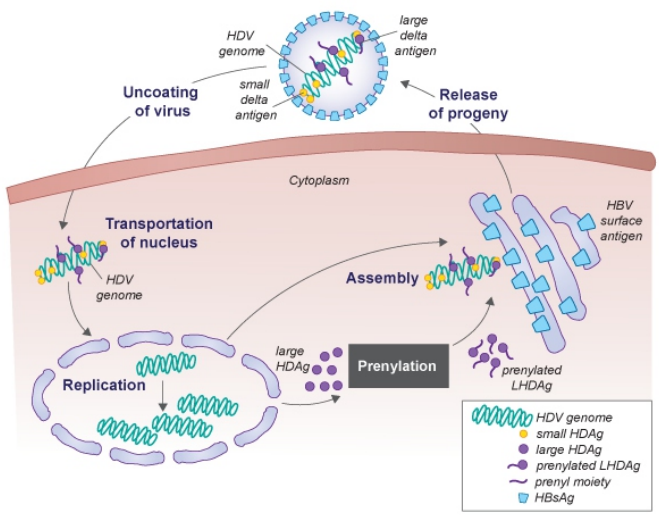
Interferons

Class Summary

These are naturally produced proteins with antiviral, antitumor, and immunomodulatory actions.

Interferon alfa-2a (Roferon)

Interferon alfa-2a has been used in several small studies to treat hepatitis D virus (HDV) infection. Dosages varying from 3-10 mU 3 times per week for as long as 12 months have been used. At the end of therapy, loss of HDV RNA and normalization of liver enzymes was seen in 50% of patients treated with 9 mU 3 times per week and in 21% in those treated with 3 mU. Half the responders remained in biochemical remission after the cessation of therapy, while no patients maintained a virologic response after cessation. Histologic improvement was observed in patients treated with interferon.



INTERPRETATION

UNDERSTANDING HEPATITIS B SEROLOGY

Demystify the technical jargon describing Hepatitis B blood tests. There is more than one test used to identify the Hepatitis B virus. In fact, there are quite a few, each with a slightly different implication. Often, attention to detail (like one small letter) differentiates one Hepatitis B test from another. To help clarify any confusion and reduce intimidation from the terminology, the most common tests appearing in a Hepatitis B blood panel are described in as simple terms as possible. Hepatitis B virus tests check for substances in the blood that show an active or past Hepatitis B infection. More specifically, serology is a test that detects the presence of antibodies against certain organisms. Antibodies, also known as immunoglobulins, are typically formed in response to an infection so that the immune system can identify and destroy that particular bacteria or virus. Thus, Hepatitis B serology is the study of the presence of antibodies in the blood for the purpose of identifying and further understanding Hepatitis B infection. When it comes to Hepatitis B, there are several different things to test for. Most Hepatitis B serology evaluates signs of infection via antibodies or antigens.

- ***Antibodies*** – As previously described, antibodies are proteins produced by the body to fight infection. The presence of Hepatitis B antibodies means that you have been exposed to this virus at some point in time.
- ***Antigens*** – Antigens are made by bacteria or viruses and they indicate that the virus is present in the body. Antigens cause the immune system to produce antibodies against it.

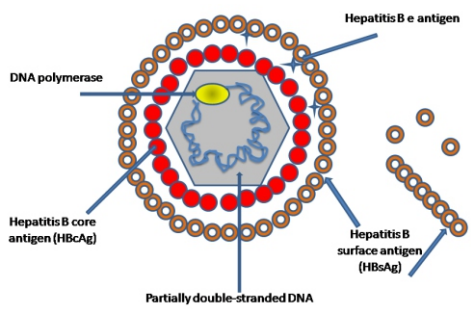
Because the name of each test is a mouthful, they are typically referred to by their acronym. However, those interested in Hepatitis B serology must pay close attention because the acronyms are extremely similar. The six serological markers for Hepatitis B include:

1. ***Hepatitis B surface antigen*** – Shortened to HBsAg, the Hepatitis B surface antigen is a protein on the surface of the virus. HBsAg can be detected in high levels in the blood during acute or chronic Hepatitis B infection. The presence of HBsAg indicates that the person is infectious. HBsAg is the antigen used to make Hepatitis B vaccine. This is the first detectable marker of infection and is present as early as the incubation period.
2. ***Hepatitis B surface antibody*** – Shortened to anti-HBs, the presence of Hepatitis B surface antibodies indicates the person has recovered from and has immunity to Hepatitis B. Anti-HBs also develops in a person who has been successfully vaccinated against Hepatitis B.
3. ***Total Hepatitis B core antibody*** – Shortened to anti-HBc, this serological marker appears at the onset of symptoms in acute Hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with Hepatitis B in an undefined timeframe.
4. ***IgM antibody to Hepatitis B core antigen*** – Shortened to IgM anti-HBc, testing positive for IgM antibody to Hepatitis B core antigen indicates a recent, acute infection with the virus.
5. ***Hepatitis B envelope antigen*** – Shortened to HBeAg, Hepatitis B envelope antigen is found in the blood during acute and chronic Hepatitis B infection. A positive HBeAg test indicates that the virus is replicating and the infected person has high levels of the Hepatitis B virus.
6. ***Hepatitis B envelope antibody*** – Shortened to HBeAb or anti-HBe, this substance is produced by the immune system temporarily during acute HBV infection or consistently during or after a burst in viral replication. A person who converts from positive HBeAg to HBeAB is more likely to achieve long-term clearance of the virus.

You can garner a lot of information about a person's Hepatitis B from their serology. Different combinations of positive and negative results for these six serological markers have very different implications. Below is a description of some of the possible combinations and what they mean:

- ***Does not have Hepatitis B immunity*** – This describes someone who has not been infected, but is still at risk for possible future infection. These individuals have negative HBsAg, negative anti-HBc and negative anti-HBs; and they are encouraged to get the vaccine.
- ***Immunity from Hepatitis B infection*** – This person has surface antibodies present due to recovery from a prior Hepatitis B infection. These individuals have negative HBsAg, positive anti-HBc and positive anti-HBs.
- ***Immunity from Hepatitis B vaccine*** – This person has surface antibodies present due to the Hepatitis B vaccination. These individuals have negative HBsAg, negative anti-HBc and positive anti-HBs.
- ***Acute Hepatitis B infection*** – Someone with an acute Hepatitis B infection has positive HBsAg, positive anti-HBc, positive IgM anti-HBc and negative anti-HBs.
- ***Chronic Hepatitis B infection*** – An estimated10 percent of people with the virus develop chronic Hepatitis B. They have positive HBsAg, positive anti-HBc, negative IgM anti-HBc and negative anti-HBs.

Although the scenarios above do not include every possible combination of Hepatitis B serology results, they demonstrate how much information can be extracted from these serological markers. Through understanding the difference between antibodies and antigens and taking the time to interpret each letter in a Hepatitis B serology test, the seeming jumble of acronyms describing Hepatitis B tests lose their ability to confuse and intimidate.



Structure of Hepatitis B

TROUBLESHOOTING

ELISA troubleshooting tips

Poor standard curve

Cause	Solution
Improper standard solution	Confirm dilutions are made correctly.
Standard improperly reconstituted	Briefly spin vial before opening; inspect for undissolved material after reconstituting.
Standard degraded	Store and handle standard as recommended.
Curve doesn't fit scale	Try plotting using different scales e.g. log-log, 5 parameter logistic curve fit.
Pipetting error	Use calibrated pipettes and proper pipetting technique.

No signal

Cause	Solution
Incubation time too short	Incubate samples overnight at 4°C or follow the manufacturer guidelines.
Target present below detection limits of assay	Decrease dilution factor or concentrate samples.
Incompatible sample type	Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect a positive control.
Recognition of epitope impeded by adsorption to plate	To enhance detection of a peptide by direct or indirect ELISA, conjugate peptide to a large carrier protein before coating onto the microtiter plate.
Assay buffer compatibility	Ensure assay buffer is compatible with target of interest (e.g. enzymatic activity retained, protein interactions retained).
Not enough detection reagent	Increase concentration or amount of detection reagent, following manufacturer guidelines.
Sample prepared incorrectly	Ensure proper sample preparation/dilution. Samples may be incompatible with microtiter plate assay format.
Insufficient antibody	Try different concentrations/dilutions of antibody.
Incubation temperature too low	Ensure the incubations are carried out at the correct temperature. All reagents including plate should be at room temperature or as recommended by the manufacturer before proceeding.
Incorrect wavelength	Verify the wavelength and read plate again.
Plate washings too vigorous	Check and ensure correct pressure in automatic wash system. Pipette wash buffer gently if washes are done manually.
Wells dried out	Do not allow wells to become dry once the assay has started. Cover the plate using sealing film or tape for all incubations.
Slow color development of enzymatic reaction	Prepare substrate solution immediately before use. Ensure the stock solution has not expired and is not contaminated. Allow longer incubation.

Large coefficient of variation (CV)

Cause	Solution
Bubbles in wells	Ensure no bubbles are present prior to reading plate.
Wells not washed equally/thoroughly	Check that all ports of the plate washer are unobstructed. Wash wells as recommended.
Incomplete reagent mixing	Ensure all reagents are mixed thoroughly.
Inconsistent pipetting	Use calibrated pipettes and proper technique to ensure accurate pipetting.
Edge effects	Ensure the plate and all reagents are at room temperature.
Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaw cycles).

High background

Cause	Solution
Wells are insufficiently washed	Wash wells as per protocol recommendations.
Contaminated wash buffer	Prepare fresh water buffer.
Too much detection reagent	Ensure the reagent has been diluted properly or decrease the recommended concentration of detection reagent.
Blocking buffer ineffective (e.g. detection reagent binds blocker; wells not completely blocked)	Try different blocking reagent and/or add blocking reagent to wash buffer.
Salt concentration of incubation/wash buffers	Increasing salt concentrations may reduce non-specific and/or weak off-target interactions.
Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution.
Non-specific binding of antibody	Use suitable blocking buffers e.g. BSA or 5-10% normal serum - species same as primary antibody if using a directly conjugated detection antibody or same as secondary if using conjugated secondary. Ensure wells are pre-processed to prevent non-specific attachment.
High antibody concentration	Try different dilutions for optimal results.
Substrate incubation carried out in light	Substrate incubations should be carried out in the dark or as recommended by manufacturer.
Precipitate formed in wells upon substrate addition	Increase dilution factor of sample or decrease concentration of substrate.
Dirty plate	Clean the plate bottom.

Low sensitivity

Cause	Solution
Improper storage of ELISA kit	Store all reagents as recommended. Please note that all reagents may not have identical storage requirements.
Not enough target	Concentrate sample or reduce sample dilution.
Inactive detection reagent	Ensure reporter enzyme/fluor has the expected activity.
Plate reader settings incorrect	Ensure plate reader is set to read the correct absorbance wavelength or excitation/emission wavelengths for fluorescent detection.
Assay format not sensitive enough	Switch to a more sensitive detection system (e.g. colorimeteric to chemiluminescence / fluorescence). Switch to a more sensitive assay type (e.g. direct ELISA to sandwich ELISA). Lengthen incubation times or increase temperature.
Target poorly adsorbs to microtiter plate	Covalently link target to microtiter plate.
Not enough substrate	Add more substrate.
Incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types. Include a sample that the assay is known to detect as a positive control.
Interfering buffers or sample ingredients	Check reagents for any interfering chemicals. For example, sodium azide in antibodies inhibit HRP enzyme and EDTA used as anticoagulent for plasma collection inhibits enzymatic reactions.
Mixing or substituting reagents from different kits	Avoid mixing components from different kits.

Matrix effect

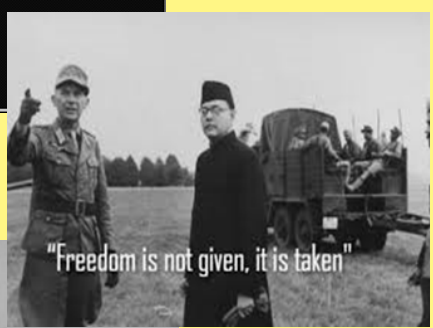
ELISA quantification of plasma and serum occasionally encounters problems which are caused by the matrix effect. The matrix effect can arise from a number of matrix components including, but not limited to: interaction between endogenous biological components such as phospholipids, carbohydrates and endogenous metabolites (bilirubin) or an interaction between the analyte of interest and the matrix, such as covalent binding to plasma proteins. This results in erroneous sample readings.

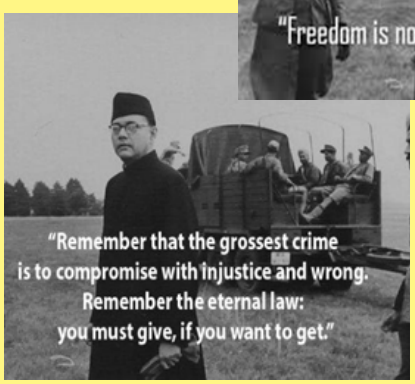
Simply diluting the samples by 2 or 5 folds reduces the matrix effect, when diluting the samples remember to use the same diluent as used for standard curve.

BOUQUET

In Lighter Vein

Wisdom Whispers





Brain Teasers

1. **False positive Widal reactions may be seen in _____.**
   - A. Past history of immunization
   - B. Inapparent infection
   - C. Anamnestic response to other vaccines
   - D. Any of the above.

2. **False negative Widal reactions may be seen in _____.**
   - A. Infection is in very early stages
   - B. Patients on antibiotic therapy while testing is conducted
   - C. Insufficient serum could give postzone effect
   - D. Any of the above.

3. **Which of the following vectors spread dengue virus?**
   - A. Aedes species
   - B. Anopheles species
   - C. Culex species
   - D. All of the above.

4. **Heterophile antibodies are associated with_____.**
   - A. Dengue fever
   - B. Infectious mononucleosis
   - C. Typhoid
   - D. Leptospirosis.

ANSWER: 1. D, 2. D, 3. A, 4. B

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