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The Crux

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Editorial

VIRAL HEMORRHAGIC FEVERS

WITH THE ONSET OF KAYSANUR FOREST DISEASE (MONKEY FEVER) AND ZIKA VIRUS FEVER, WE HAVE BEEN REQUESTED TO COMMENT ON CLINICO-DIAGNOSTICS OF VHFs. SO HERE IT GOES..........UNDER THE SECTION "DISEASE DIAGNOSIS"

The **viral hemorrhagic** (or **haemorrhagic**) **fevers** (**VHFs**) are a diverse group of animal and human illnesses in which fever and hemorrhage are caused by a viral infection. VHFs may be caused by five distinct families of RNA viruses: the families *Arenaviridae*, *Filoviridae*, *Bunyaviridae*, *Flaviviridae*, and *Rhabdoviridae*. All types of VHF are characterized by fever and bleeding disorders and all can progress to high fever, shock and death in many cases. Some of the VHF agents cause relatively mild illnesses, such as the Scandinavian *nephropathia epidemica* (a Hantavirus), while others, such as Ebola virus, can cause severe, life-threatening disease.

Five families of RNA viruses have been recognised as being able to cause hemorrhagic fevers.

- The family ***Arenaviridae*** include the viruses responsible for Lassa fever (Lassa virus), Lujo virus, Argentine (Junin virus), Bolivian (Machupo virus), Brazilian (Sabiá virus) and Venezuelan (Guanarito virus) hemorrhagic fevers.
- The family ***Bunyaviridae*** include the members of the *Hantavirus* genus that cause hemorrhagic fever with renal syndrome (HFRS), the Crimean-Congo hemorrhagic fever (CCHF) virus from the *Nairovirus* genus, Garissa virus and Ilesha virus from the *Orthobunyavirus* and the Rift Valley fever (RVF) virus from the *Phlebovirus* genus.
- The family ***Filoviridae*** include Ebola virus and Marburg virus.
- The family ***Flaviviridae*** include dengue, yellow fever, and two viruses in the tick-borne encephalitis group that cause VHF: Omsk hemorrhagic fever virus and Kyasanur Forest disease virus.
- In September 2012 scientists writing in the journal PLOS Pathogens reported the isolation of a member of the ***Rhabdoviridae*** responsible for 2 fatal and 2 non-fatal cases of hemorrhagic fever in the Bas-Congo district of the Democratic Republic of Congo. The non-fatal cases occurred in healthcare workers involved in the treatment of the other two, suggesting the possibility of person-to-person transmission. This virus appears to be unrelated to previously known Rhabdoviruses. Flip this page, read on..........

INTERPRETATION highlights the problems faced while interpreting Widal Test. All aspects are covered in an easily assimilable format.

TROUBLESHOOTING shoots down the troubles encountered in the procedural protocols in PCR tests. Though methods may vary from company to company.

Pictures have taken over BOUQUET and rightly so!

Tulip Group

DISEASE DIAGNOSIS

VIRAL HEMORRHAGIC FEVERS

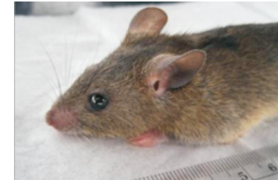
Background

Viral hemorrhagic fevers (VHFs) are a group of febrile illnesses caused by RNA viruses from several viral families. These highly infectious viruses lead to a potentially lethal disease syndrome characterized by fever, malaise, vomiting, mucosal and gastrointestinal (GI) bleeding, edema, and hypotension. The four viral families known to cause VHF disease in humans include the Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. General characteristics of these viral families can be found in the table below. The image below depicts palatal petechiae and hemorrhage in a patient with Ebola hemorrhagic fever.

Filovirus disease - Ebola fever. Patient with Ebola hemorrhagic fever during 1976 outbreak in Zaire demonstrating palatal petechiae and hemorrhage.

Arenaviridae

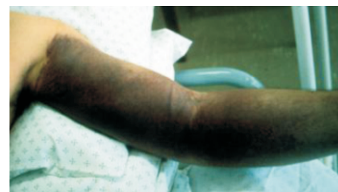
Arenaviridae are spread to humans by rodent contact and include Lassa virus in Africa and several rare South American hemorrhagic fevers such as Machupo, Junin, Guanarito, and Sabia. Lassa virus is the most clinically significant of the Arenaviridae, accounting for serious morbidity and mortality in West Africa. Lassa fever first appeared in Lassa, Nigeria, in 1969. It has been found in all countries of West Africa and is a significant public health problem in endemic areas. In populations studied, Lassa fever accounts for 5-14% of hospitalized febrile illnesses. Its natural reservoir is a small rodent whose virus-containing excreta is the source of transmission.



Mastomys natalensis, natural host of Lassa virus.

Bunyaviridae

This group includes Rift Valley fever (RVF) virus, Crimean-Congo hemorrhagic fever (CCHF) virus, and several hantaviruses. The RVF and CCHF viruses are both arthropod-borne viruses. RVF virus, an important African pathogen, is transmitted to humans and livestock by mosquitos and by the slaughter of infected livestock. CCHF virus is carried by ticks and causes a fulminant, highly pathogenic form of VHF notable for aerosol transmission of infective particles. Outbreaks of CCHF have occurred in Africa, Asia, and Europe.



Bunyavirus infection. Ecchymoses encompassing left upper extremity one week after onset of CCHF. Ecchymoses often are accompanied by hemorrhage in other locations: epistaxis, puncture sites, hematemesis, melena, and hematuria.

Table. Viral Families Causing Viral Hemorrhagic Fever

Virus Family	Disease (Virus)	Natural Distribution	Usual Source of Human Infection	Incubation (Days)
Arenaviridae				
Arenavirus	Lassa fever	Africa	Rodent	5-16
	Argentine HF (Junin)	South America	Rodent	7-14
	Bolivian HF (Machupo)	South America	Rodent	9-15
	Brazilian HF (Sabia)	South America	Rodent	7-14
	Venezuelan HF (Guanarito)	South America	Rodent	7-14
Bunyaviridae				
Phlebovirus	Rift Valley fever	Africa	Mosquito	2-5
Nairovirus	Crimean-Congo HF	Europe, Asia, Africa	Tick	3-12
Hantavirus	Hemorrhagic fever with renal syndrome, hantavirus pulmonary syndrome	Asia, Europe, worldwide	Rodent	9-35
Filoviridae				
Filovirus	Marburg and Ebola	Africa	Fruit bat	3-16
Flaviviridae				
Flavivirus	Yellow fever	Tropical Africa, South America	Mosquito	3-6
	Dengue HF	Asia, Americas, Africa	Mosquito	Unknown for dengue HF, 3-5 for dengue

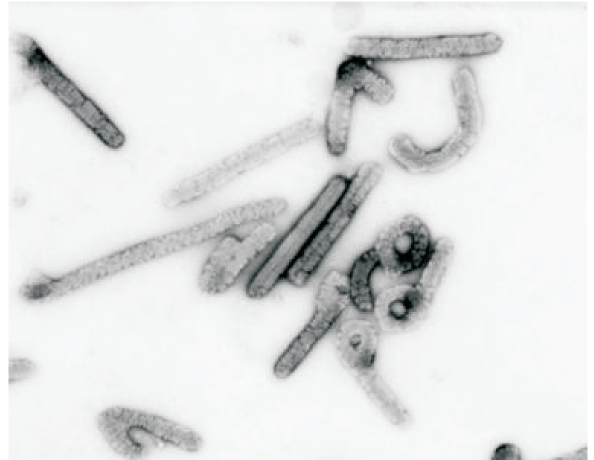
Many hantaviruses are spread worldwide, causing 2 major syndromes: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). They are divided into Old World hantaviruses (such as the prototypical Hantaan virus of Korea), which generally cause HFRS, and New World hantaviruses, causing HPS. Rodents carry both types. A previously undiscovered Hantavirus, Sin Nombre virus, was the cause of an outbreak of highly lethal HPS in the southwestern US in 1993. More than 450 cases have been identified in the US since 1993 with a 35% mortality rate.

Filoviridae

The most notorious of the VHF viruses, including Ebola and Marburg viruses, belong to the Filoviridae family. Ebola virus first was described in 1976 after outbreaks of a febrile, rapidly fatal hemorrhagic illness were reported along the Ebola River in Zaire (now the Democratic Republic of the Congo) and Sudan. Sporadic outbreaks have continued since that time, usually in isolated areas of central Africa. An outbreak in Kikwit, Zaire, in 1995 led to 317 confirmed cases, with an 81% mortality rate. Two thirds of the patients were among health care workers caring for infected individuals. An outbreak in Uganda in late 2000 resulted in 425 cases and claimed 225 lives. In late 2012, 7 cases of Ebola virus infection were reported, including 4 deaths Ebola has 4 distinct subtypes: Ebola-Zaire, Ebola-Sudan, Ebola-Ivory Coast, and Ebola-Reston, a form that causes illness in nonhuman primates, and as has been recently discovered, in pigs.A 2007 Ebola outbreak in Uganda, however, has been attributed to a new form of Ebola. This new Ebola subtype, which appears to be closely related to Ebola-Ivory Coast, has been given the proposed name Bundibugyo Ebola virus (named after the Bundibugyo district in Western Uganda). The most recent outbreakin western africa has killed thousands of people. Fruit bats have been identified as a reservoir for Ebola-Zaire virus.

Ebola virus. Electron micrograph

Marburg virus, named after the German town where it first was reported in 1967, is another highly pathogenic member of the Filoviridae family that is traced to central Africa. As in Ebola-Zaire, the natural host for the virus is likely the fruit bat. Marburg virus was contracted by a traveler to central Africa in 1987 and has been endemic since 1998 in Durba, Democratic Republic of the Congo, and in persons exposed in caves or mines. Marburg virus was determined to be the causative agent in a 2004-2005 outbreak of hemorrhagic fever in Angola that led to 252 confirmed cases and 227 deaths (90% case-fatality rate). In late 2012, an outbreak in Uganda resulted in 15 confirmed and 8 probable cases of Marburg virus infection, including 15 deaths.



Marburg virus. Negative stain image

Flaviviridae

Yellow fever and dengue fever are the most well known diseases caused by flaviviruses. Both are mosquito-borne; yellow fever is found in tropical Africa and South America, and dengue fever is found in Asia, Africa, and the Americas. They are notable for their significant effect on prior military campaigns and their continued presence throughout endemic areas. Due to a resurgence in the last 3 decades, dengue fever is now considered second only to malaria in terms of importance as a tropical disease. Multiple recent large outbreaks have occurred throughout the tropics, with the most severe outbreaks occurring in Southeast Asia and the western Pacific regions. Transmission is via the bite of the infected female Aedes mosquito, although dengue can also be transmitted via transfusion.

Pathophysiology

The primary defect in patients with viral hemorrhagic fever (VHF) is that of increased vascular permeability. Hemorrhagic fever viruses have an affinity for the vascular system, leading initially to signs such as flushing, conjunctival injection, and petechial hemorrhages, usually associated with fever and myalgias. Later, frank mucous membrane hemorrhage may occur, with accompanying hypotension, shock, and circulatory collapse. The relative severity of the clinical presentation may vary depending on the virus in question, amount, and route of exposure. In acute disease, patients are extremely viremic, and messenger ribonucleic acid (mRNA) evidence of multiple cytokine activation exists. In vitro studies reveal these cytokines lead to shock and increased vascular permeability, the basic pathophysiologic processes most often seen in viral hemorrhagic fever infection. Another prominent pathologic feature is pronounced macrophage involvement. Inadequate or delayed immune response to these novel viral antigens may lead to rapid development of overwhelming viremia. Extensive infection and necrosis of affected organs also are described. Hemorrhagic complications are multifactorial and are related to hepatic damage, consumptive coagulopathy, and primary marrow injury to megakaryocytes. Aerosol transmission of some viral hemorrhagic fever infections is reported among nonhuman primates and likely is a mode of transmission in patients with severe infection. Multisystem organ failure affecting the hematopoietic, neurologic, and pulmonary systems often accompanies the vascular involvement. Hepatic involvement varies with the infecting organism and is at times seen with Ebola, Marburg, RVF, CCHF, and yellow fever. Renal failure with oliguria is a prominent feature of HFRS

seen in Hantavirus infection and may be seen in other VHFs as intravascular volume depletion becomes more pronounced. Bleeding complications are particularly prominent with Ebola, Marburg, CCHF, and the South American arenaviruses. Although the pathophysiology of dengue infection is complex and incompletely understood, severe dengue infection can be differentiated from milder forms by the presence of increased vascular permeability. The greatest risk factor for severe dengue infection is secondary infection with a dengue serotype different from the initial dengue infection. This increased vascular permeability is thought to be secondary to widespread T-cell activation and apoptosis and is also thought to be related to a process known as antibody-dependent enhancement, best described as the balance between neutralizing versus enhancing antibodies after an initial dengue infection, which can contribute to the severity of secondary dengue infection.

Epidemiology

Frequency

International: Lassa fever is responsible for an estimated 100,000-300,000 infections per year, with 5,000 deaths. Cases have been reported throughout West Africa, particularly in Nigeria, Sierra Leone, Guinea, and Liberia. Other arenaviruses are responsible for sporadic VHF outbreaks throughout South America. Rift Valley fever (RVF) virus and Crimean-Congo hemorrhagic fever (CCHF) are responsible for intermittent epidemics in Africa (for RVF) and in areas of Africa, Asia, and Europe (for CCHF). HFRS due to Hantavirus infection continues to be an ongoing health concern, particularly in Asia, affecting up to 200,000 patients annually. Ebola virus appears sporadically in endemic areas of the former Zaire and Sudan. Ebola virus also has been reported in Gabon, the Ivory Coast, and Uganda. Outbreaks appear to propagate in hospital settings, often involving health care providers. Yellow fever continues to be a serious problem in tropical areas of South America and Africa, where vaccination is not widespread. The World Health Organization estimates that approximately 200,000 cases per year occur in Africa. Dengue HF is endemic in Southeast Asia, Africa, Central America, and South America, and the WHO estimates that 50-100 million cases occur annually. In 2012, Rio de Janeiro recorded more than 180,000 cases of dengue infection.

Dengue Virus Notice posted outside Maracanã Stadium, Rio de Janeiro, Brazil, 2012.
Translation: This site is a strategic point for controlling Dengue.

Mortality/Morbidity

Case-fatality rates of patients with VHF vary from less than 10% (eg, in dengue HF) to approximately 90%, as has been reported in patients with Ebola-Zaire and the recent Angola Marburg outbreak. The most recent outbreak of Ebola-Sudan in Uganda had a 50% case-fatality rate. Complications from VHF infection include retinitis, orchitis, hepatitis, transverse myelitis, and uveitis. In patients who recover from Lassa fever infection, deafness is the most common complication. Spontaneous abortion also is common. Renal insufficiency is associated with HFRS infection. **Race:** No racial predilection has been identified, although cases have originated in African areas. **Sex:** No predilection for either sex has been identified. **Age:** VHF affects all ages according to exposure and local demographics.

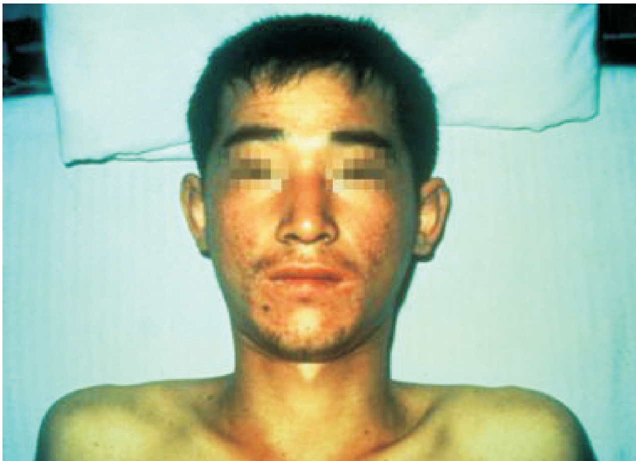
History

Obtain a detailed travel history, paying particular attention to recent travel to tropical or rural areas, such as Central or South America (yellow fever, arenaviruses), West Africa (Lassa fever), or to endemic portions of Central Africa (Ebola, Marburg, RVF, CCHF). Ask about contact with potential arthropod or rodent reservoirs. Since the natural reservoir for Ebola and Marburg viruses is unknown, contact with infected monkeys or humans is not a prerequisite for transmission of infection. Direct contact with rodents infected with hemorrhagic fever viruses (eg, arenaviruses, hantaviruses) is not necessary for transmission of infection, since aerosolized excreta may transmit infection. Contacts of patients with known viral hemorrhagic fever (VHF), especially family members or health care workers caring for infected patients, are at risk for infection if appropriate barrier precautions are not used. Transmission of VHF has occurred from the reuse of unsterile needles and syringes used for treatment of infected patients. Transmission of VHF also has occurred to individuals handling the deceased in preparation for burial or to individuals involved in the slaughter of infected livestock (as in RVF or CCHF). Because of their extreme pathogenicity and potential for transmission by fine-particle aerosol, VHF viruses are considered potential biological warfare agents. In addition, Dr Ken Alibek, the former Deputy Director of the once massive Soviet bioweapons program, Biopreparat, claims Soviet scientists successfully had produced a stable Marburg virus biological weapon that could be delivered as an aerosol. Large numbers of military personnel with VHF symptoms would suggest such an attack. An outbreak of VHF in a nonendemic area would also suggest a biological warfare attack. Incubation periods for VHF vary from 2-21 days. The initial symptoms correspond to development of viremia and include the following: High fever, Headache, Fatigue, Abdominal pain, Myalgias, Prostration. In more advanced disease, signs and symptoms include the following: Hematemesis and bloody diarrhea, Generalized mucous membrane hemorrhage, Rash, Altered mental status and cardiovascular collapse (preterminal events).

Physical

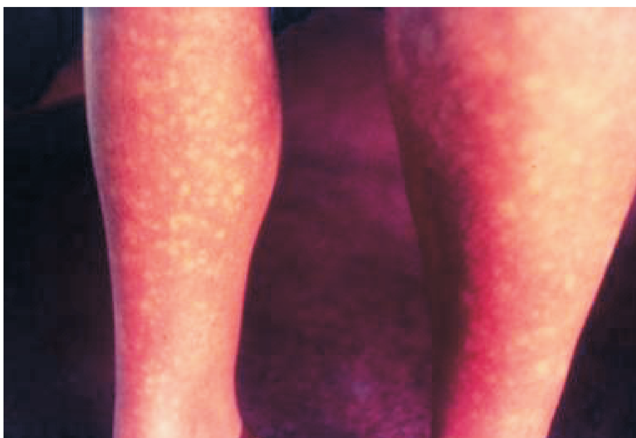
Depending on the progress of the disease, patients with viral hemorrhagic fever (VHF) initially may present with minimal signs, suggesting a more benign viral syndrome. Maintain a high index of suspicion. As the disease progresses, more classic findings are present as follows: Fever, Pharyngitis, Conjunctival injection, Nondependent edema, Petechial or ecchymotic rash, GI bleeding, Hypotension and/or shock. Most hemorrhagic fevers, except Rift Valley fever, can produce a variety of cutaneous findings that are principally caused by vascular instability and bleeding abnormalities. Such findings include flushing, petechiae, purpura, ecchymoses, and edema. The Old World arenavirus

causing Lassa fever results in the greatest amount of edema of any of the hemorrhagic fever viruses. Additionally, no bleeding abnormalities are present. The New World arenaviruses (Junin, Machupo, Sabia, and Guanarito) cause less edema and variable amounts of petechiae, purpura, ecchymoses, palatal hyperemia, and mucosal hemorrhage. The most severe hemorrhage from a hemorrhagic fever virus follows infection with the Congo Crimean hemorrhagic fever virus. Hantaviruses can cause a relatively distinctive eruption with a petechial eruption around the neck and on the anterior and posterior axillary folds, arms, and trunk. A sunburn-like flush is seen on the head, neck, and upper chest and back and may be accompanied by facial edema (see the image below). Sometimes, a morbilliform eruption occurs. Oral and conjunctival surfaces may develop severe hemorrhages.



Bunyavirus infection - Hantaan virus. Patient with Korean hemorrhagic fever caused by Hantaan virus demonstrating typical 'sunburn flush' of cheeks, chin, and base of neck. Photo courtesy of John Huggins, PhD.

The greatly feared filoviruses (Marburg and Ebola) exhibit characteristic exanthems that are best seen in fair-skinned patients. Soft palatal hyperemia accompanies the flu-like prodrome and is followed between days 5 and 7 by a nonpruritic, centripetal, pinhead-sized papular, erythematous exanthem. Within 24 hours, this can develop into large and coalescent, well-demarcated, sometimes hemorrhagic macules and papules. In severe cases, hemorrhage exudes from mucous membranes, venipuncture sites, and body orifices. Dengue virus causes a characteristic erythematous exanthem with striking islands of sparing (see the image below).



Patient with morbilliform exanthem of dengue fever. Note islands of sparing characteristics for dengue.

Diagnostic Considerations

Other problems to be considered in the differential diagnosis include the following: Typhoid fever, Shigellosis, Meningococcemia, Rickettsial infections, Acute leukemia, Idiopathic or thrombotic thrombocytopenic purpura.

Differential Diagnoses

Disseminated Intravascular Coagulation, Emergent Management of Malaria, Hemolytic Uremic Syndrome in Emergency Medicine, Leptospirosis in Humans, Relapsing Fever in Emergency Medicine, Salmonella Infection in Emergency Medicine, Systemic Lupus Erythematosus (SLE), Thrombotic Thrombocytopenic Purpura.

Laboratory Studies

Because of risks associated with handling infectious materials, perform the minimum necessary laboratory testing for diagnostic evaluation and patient care. Considerations in ordering lab tests are as follows: A complete blood count often indicates leukopenia and thrombocytopenia (these findings may not be present in Lassa fever). Elevated hepatic transaminases are observed in viral hemorrhagic fever (VHF) and are predictive of high mortality in Lassa fever infection. Prothrombin time, activated partial thromboplastin time, international normalized ratio, and clotting times are prolonged. A disseminated intravascular coagulation profile including fibrinogen level, fibrin degradation products, and platelet count may be useful.

Other Tests

Most patients are viremic at the time of presentation (Hantavirus is an exception). Specific viral diagnosis can be made using serologic tests, including enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction. Difficult cases may require tissue cultures. During the 2000-2001 Ebola outbreak in Uganda, reverse transcriptase-PCR (RT-PCR) emerged as a very effective means for detecting Ebola virus in patient serum, plasma, and whole blood. Each nation has specific centers for appropriate diagnostic protocols. These facilities should have Biosafety Level 4 facilities. Report all suspected cases of viral hemorrhagic fever (VHF) immediately to local and state public health departments.

Prehospital Care

Supportive care is based on the patient's physiologic condition. Because most patients requiring prehospital evaluation and transport are in the early stages of the disease, universal precautions should be adequate. In patients with respiratory symptoms (eg, cough, rhinitis), use face shields and high-efficiency particulate air (HEPA) filter masks.

Emergency Department Care

Fluid resuscitation and supportive care are the mainstays of emergency department therapy. Intravenous crystalloids, oxygen, and cardiac monitoring are the most appropriate initial steps in the treatment of patients in whom viral hemorrhagic fever (VHF) is suggested. Other measures include the following: Administer blood and blood products as clinically indicated. Avoid intramuscular injections and the use of aspirin or other anticoagulants. Minimize invasive procedures because of the risk associated with viral transmission from sharp objects. Infection control measures include the following: Place patients in a private room. A negative pressure room is not necessary during early stages of the

disease but may be necessary if patients have prominent cough, vomiting, diarrhea, or hemorrhage. Prevent nonessential staff and visitors from entering the room. All staff entering the room should wear gloves and gowns. Persons coming within 3 feet of the patient should wear face shields or surgical masks with eye protection (including side shields); use HEPA filter masks if patients have prominent respiratory, GI, or hemorrhagic symptoms. If large amounts of blood or other body fluids are present in the environment, use leg and shoe coverings. Before exiting the room, discard all used protective barriers and clean shoes with a hospital disinfectant or solution of household bleach. If possible, use an anteroom for putting on and removing protective barriers and for storing supplies.

Medication Summary

No specific antiviral therapy is available for Ebola or Marburg virus infection. The use of convalescent serum (ie, sera from patients who have survived infection) is suggested as a possible therapy. Late during the 1995 Kikwit, Zaire, outbreak, 8 Ebola patients received blood transfusions from Ebola survivors. Of these, 7 survived. However, no clear evidence exists that links their survival directly to this therapy. More recent efforts have focused on viral inhibition, including Ebola virus inhibition using selective estrogen receptor modulators. Lassa fever and HFRS due to Hantavirus infection have been treated effectively with intravenous and oral ribavirin. Because of this, ribavirin has been recommended as a potential treatment for other arenaviruses and bunyaviruses. Treatment is most effective when given early in the clinical course. Ribavirin also is recommended for postexposure prophylaxis. Other potential antiviral therapies against Lassa fever include novel benzimidazole compounds such as ST-193 and other related heterocyclic compounds. Recently proposed guidelines for the use of ribavirin for postexposure prophylaxis recommend the use of oral ribavirin exclusively for definitive, high-risk exposures, such as contaminated needlestick injury, mucous membrane or nonintact skin exposure with contaminated blood or body fluids, participation in emergency resuscitative procedures (eg, intubation, suctioning), or prolonged close contact in an enclosed space with infected patients without appropriate personal protective equipment. Recent research into the development of antiarenaviral drugs has focused on broad screening of small molecules with potential antiviral activity. This high-throughput screening (HTS) strategy has previously identified antiviral drugs and may potentially provide novel inhibitors of viral cell entry in the future. Development of a Lassa virus vaccine is continuing at the CDC. Yellow fever vaccine is readily available and is both safe and effective. A bivalent vaccine is being developed from the preexisting 17D yellow fever vaccine that would express not only yellow fever glycoproteins but also Lassa glycoproteins, theoretically stimulating a protective immune response against both viruses. A recent study evaluating the safety and efficacy of a tetravalent dengue vaccine demonstrated full seroconversion against all WHO dengue serotypes in flavivirus-naive adults. Argentine HF (Junin) vaccine is also effective and may protect against Bolivian HF as well. Rift Valley fever and Hantaan (HFRS) vaccines are also available. Although there is no approved vaccine for either Ebola or Marburg virus, significant progress has been made in developing an effective experimental vaccine using a vesicular stomatitis virus-based vaccine. In March 2009, after a German researcher sustained a needlestick while working with Ebola virus, a decision was made among Ebola experts and researchers to administer an experimental Ebola vaccine that has shown effectiveness in nonhuman primate studies. Initial reports indicate that this effort may have been successful. Other recent efforts have focused on postexposure prophylaxis for filovirus exposure and have achieved success using a primate model. Other efforts to create a viable (and marketable) Ebola vaccine have led to the development of an experimental bivalent vaccine that confers protection against both rabies and Ebola virus.

Class Summary

The goals in the use of antivirals are to shorten the clinical course, prevent complications, prevent the development of latency and/or subsequent recurrences, decrease transmission, and eliminate established latency.

Ribavirin (Virazole)

Nucleoside analog with antiviral activity; may significantly reduce mortality in Lassa fever and Hantavirus infection if treatment begun within 6 d of onset.

Further Inpatient Care

Hospitalize patients with suspected or confirmed viral hemorrhagic fever (VHF) infection because of the significant risk for nosocomial spread of the infection. Notification of local and state public health departments may provide resources for further epidemiologic investigation into the source of the infection. Appropriate barrier precautions should remain in place throughout the hospital course because of the highly pathogenic nature of viral hemorrhagic fever infection and because various causes of viral hemorrhagic fever often are clinically indistinguishable.

Deterrence/Prevention

As the natural reservoirs for Ebola and Marburg virus infection remain unknown, no specific prevention measures are established. Recent studies have suggested that contact with fruits bats may be responsible for some cases of filovirus infection. Efforts are under way in West Africa to educate people in high-risk areas about ways to decrease rodent populations, thereby reducing transmission of Lassa fever. Strict barrier precautions in the treatment of patients with known or suspected viral hemorrhagic fever infection reduce nosocomial transmission.

Complications

Complications from viral hemorrhagic fever (VHF) infection include retinitis, orchitis, encephalitis, hepatitis, transverse myelitis, and uveitis. In patients who recover from Lassa fever infection, deafness is the most common complication. Spontaneous abortion also is common. Renal insufficiency is associated with HFRS infection.

INTERPRETATION

WIDAL TEST

Agglutination is a classic serologic reaction that results in clumping of a cell suspension by a specific antibody, directed against a specific antigen. Such tests have been widely used for detection of antibodies against various disease-producing micro-organisms in serum for a long time. The Widal agglutination test, developed by F Widal in 1896[1] to aid in the diagnosis of typhoid fever, utilises a suspension of killed *Salmonella typhi* as antigen, to detect typhoid fever in serum from suspected *S typhi*-infected patients who present with febrile illness. The value and clinical application of the Widal test in developed countries has diminished considerably in recent years[2] and a large number of antigenically related determinants of both typhoid and non-typhoid *Salmonella* organisms are now recognised. We therefore decided to review the significance of this sero-diagnostic test for typhoid fever in modern medicine, and to discuss new and innovative alternative diagnostic tests. Hopefully, this review will offer both the novice and the experienced physician the opportunity to appreciate the limitations of the Widal test.

Widal agglutination

Widal agglutination was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi*. While the definitive diagnosis of typhoid fever depends on the isolation of *S typhi* from blood, stools, urine or other body fluids,[3-5] the role of the Widal test had been to increase the index of suspicion for the presence of typhoid fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of a four-fold rise of antibody titre.[6-8] In developed countries, the use of Widal agglutination as a laboratory tool to aid in the diagnosis of typhoid fever during the acute phase of the illness, has largely been abandoned,[2] as the need for such a test is minimal, especially in view of the low prevalence of typhoid fever. In addition, adequate and improved sanitation, sewage systems, proper hygiene and better means of isolating the organism from culture are available. Unfortunately, in some developing countries, the situation is quite different, and the Widal test appears to be the only laboratory means employed in the diagnosis of typhoid fever among suspected patients. As the test suffers from serious cross-reactivity with other infectious agents, it may produce false-positive results, leading to an over-diagnosis of typhoid fever. Reynolds *et al* [9] concluded that diagnosis of typhoid fever based on serology (Widal agglutination) alone is frequently inaccurate. Concomitant with this increase in diagnosis is the abuse of the first-line drug of choice (chloramphenicol), which has led to the selection of resistant strains of *S typhi*.

Performance technique

The Widal test reaction involves the use of bacterial suspensions of *S typhi* and *S paratyphi* 'A' and 'B', treated to retain only the 'O' and 'H' antigens. These antigens are employed to detect corresponding antibodies in the serum of a patient suspected of having typhoid fever. The IgM somatic O antibody appears first and represents the initial serologic response in acute typhoid fever, while the IgG flagella H antibody usually develops more slowly but persists for longer. Two types of agglutination techniques are available: the slide test and the tube test. The slide test is rapid and is used as a screening procedure. Using commercially available antigens of *S typhi*, a drop of the suspended antigen is added to an equal amount of previously prepared serum. An initial positive screening test requires the determination of the strength of the antibody. This is done by adding together equal amounts of antigen suspension and serially diluted serum from the suspected patient. Agglutinations are visualised as clumps. Weakly reactive agglutinations may require an adequate light source for proper visualisation, while strongly reactive agglutinations are easily seen. The result of the tests are scored from 0 to 4+, ie, 0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination). The smallest quantity of serum that exhibits a 2+ or 50% agglutination is considered the end-point of serum activity or titre. The tube agglutination test requires much more technical work than the rapid slide test, and is a macroscopic test. It also serves as a means of confirming the results of the slide test. A mixture of suspended antigen and antibody is incubated for up to 20 h at 37°C in a water bath. Agglutinations are visualised in the form of pellets, clumped together at the bottom of the test tube. Results are scored from 0 to 4+ positive agglutination as described above for the slide test. The tube test is useful to clarify erratic or equivocal agglutination reactions obtained by the more rapid slide test. Since the ultimate goal of the test is antigen–antibody complex reaction, cross-reactions are encountered when antibody produced by non-typhoidal antigens reacts with typhoid-specific antigens. Several other diseases caused by non-*Salmonella* organisms (malaria, dengue, miliary tuberculosis, endocarditis, chronic liver disease, brucellosis, etc) have been shown to exhibit this cross-reactivity in typhoid endemic regions, and these cross-reactions increase the error rate of the result of the Widal test. Lack of standardisation of antigens also compromises the technique. The value of Widal test depends upon the standardisation and maintenance of the antigens to produce consistent results, and it has become evident from work done in recent years on standardisation of the Widal test and interpretation of the results that both the O and H antigens are necessary for proper serologic analysis of the suspected serum. The widespread use of typhoid–paratyphoid vaccine, as well as the large number of cases of repeated exposure to *Salmonella* species, tend to lower the specificity of the Widal test. It is considered that serologic studies are helpful in typhoid fever cases in endemic regions only if patients have four-fold or greater increases in O or H agglutinin titres in serum specimens obtained 2–3 weeks apart.

Interpretation of the test results

While performance of the test may require some detailed technical work, interpreting the test result is the more arduous task. *Salmonella* are divided into distinct serologic groups (A through E) on the basis of their somatic O antigens. While all group D organisms, such as *S typhi* possess O antigen 9, about 60 of the 78 group D serotypes including *S typhi* also have O antigen 12. Thus, infection by any of the group D serotypes can produce antibodies that can react with the O antigen used in the Widal reaction. Also, since all groups A and B organisms possess O antigen 12, cross-reactions with O antibody of group D serotype can

occur with any of the group A and B serotype O antigens. Depending on the relative quality and quantity of antigenicity of the O antigens 9 and 12 contained in other common non-typhoidal *Salmonella* serotypes, cross-reaction may occur frequently enough to lessen considerably the diagnostic specificity of the Widal reaction. A negative agglutination test may be for one of several reasons, A negative Widal test result does not therefore necessarily rule out the absence of infection. Such results are best kept as a reference for subsequent comparative analysis. A positive agglutination tests (on two successive occasions) on the other hand, may also be open to several different interpretations. Although there are controversies surrounding the increase in titre beyond the first week of illness in some endemic areas, it is generally accepted by clinicians that, toward the end of the first week of illness, titres of either O or H antibody may rise to as high as 1:160. However, the lack of paired sera may lead to an erroneous interpretation of test results. In endemic typhoid regions, a single testing of a serum specimen for Widal agglutinin cannot provide a reliable diagnosis due to: repeated exposure to small inocula of *S typhi* or to other *Salmonella* spp that contain type 9 or 12 antigens, previous typhoid fever immunisation, other infectious agents such as malaria. Although a number of reports from some developing countries have suggested that a single Widal test is sufficient to make the diagnosis of typhoid fever, others have disputed the usefulness of such a single test result.. In some developing countries where the use of a single Widal test appears to be the norm, there has been an increase in the rate of false-positive results. It has been noticed that Widal agglutinin in malaria infection in a Nigerian population and found that 85% of patients with a negative *S typhi* culture but positive malaria smear had Widal titres of 1:40, 12% had titres of 1:80, and 3% had titres of 1:160. In contrast, 45% of patients with both *S typhi* cultures and malaria smears negative had Widal titres of 1:40, 15% had titres of 1:80, and 10% had titres of 1:160. Schroeder concluded in a review of clinical interpretation of serologic tests for typhoid fever that the tests are nonspecific, poorly standardised, confusing and difficult to interpret. Erroneous interpretation of the test result may lead to misdiagnosis and mismanagement of the patient, resulting in major morbidity and mortality. In interpreting Widal test results, it is important that there should be close communication between the physician requesting the test and the laboratory, since modifications of technique in individual laboratories may affect the Widal titres and some patients with bacteriologically confirmed typhoid fever may fail to develop the usual rise of antibody titres. The results of the tests should be reported as either 'no agglutination' or, if agglutination is present, in titres (1:20, 1:40 or 1:80) rather than in descriptive (negative or positive) terms, as the latter may be misleading and contribute to the false interpretation of the test result by the physician. The function of the laboratory is to perform and report the test result to the requesting physician, who in turn will use the data to help make the proper diagnosis. Unfortunately, in several areas of developing countries, the laboratory performs the test, makes the diagnosis and prescribes the antibiotics. It should be stressed that a single Widal agglutination test has no diagnostic significance. According to Hoffman *et al*, the results of a single Widal test, tube dilution, micro-agglutination or slide agglutination are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known, as well as predictive values. Even in the extreme case of a high titre in a single Widal agglutination test, the causative organism may often be due to other species of *Salmonella*, rather than *S typhi*. Sansone *et al* published a case report where the Widal reaction to typhoid O antigen on admission for an unexposed patient was 1:320, with an increase in titre to 1:20 480 by the fourth day. While both blood and urine cultures were negative for *S typhi* in this case, a non-typhoidal *Salmonella* sp was isolated from the stool of this patient which was identified as *S javiana*. In an individual with no prior exposure to *S typhi* infection (either lack of active infection or absence of passive immunisation), a higher than 1:50 or 1:100 titre on an initial single test, usually correlates fairly well with exposure to typhoid fever. However, even these single high-value titres in an endemic area where repeated exposures to *S typhi* may have occurred, do not have any clinical relevance in the absence of a positive isolate of the causative organism or its antigen.

Limitations of the Widal test

While the Widal test has played a major role in the diagnosis of typhoid fever in the past, recent technical developments have revealed several pitfalls in its use and interpretation of its result. Clinically, it is obvious that a single Widal test in an unvaccinated or unexposed patient may have some diagnostic relevance. However, the result of such a single test has no diagnostic significance in an endemic region; in part due to difficulty in establishing a steady-state or baseline titre of Widal agglutination, which limits the usefulness of the test as a reliable diagnostic indicator of the disease process. The results of studies done in Nigeria to evaluate the clinical value of a single Widal test and the presence of Widal agglutinin in malaria infection are summarised in the table. The common denominator between the two groups was the lack of prior immunisation against typhoid fever and absence of positive *S typhi* culture. One would not therefore expect any patient to have any specific Widal agglutinin in their serum, unless there are related, undetected, antigenic determinants of *S typhi* present in the cells of other organisms. The presence of Widal agglutinin under conditions of positive malaria smear, negative *S typhi* culture and negative prior typhoid immunisation, would suggest that malaria parasite may have some undefined antigenic determinants similar to *S typhi* which can induce antibody production. This could explain the febrile condition seen in some of these patients. On the other hand, the presence of Widal agglutinin under conditions of negative malaria smear, negative *S typhi* culture and negative prior immunisation against typhoid fever suggests that other infectious agents, in addition to *Salmonella* and malaria parasite, may also share common antigenic determinants with *S typhi*. These findings are in agreement with other reports from India with similar environmental and disease (malaria, typhoid) conditions, two cases from Canada, and a case from Baltimore, all of which cast further doubt on the reliability and the use of Widal test for the diagnosis of typhoid fever in endemic regions. The use of the Widal test to diagnose typhoid fever should therefore be limited to situations in which there is no other confirmatory supportive test, such as positive culture, available. Similarities between typhoidal and non-typhoidal *Salmonella* antigens mean that a serological method of diagnosis is the least accurate for typhoid fever. Due to the inexperience of some clinicians in typhoid endemic countries, many cases of pyrexia of unknown origin receive the diagnosis of typhoid fever, based upon a false-positive Widal test result rather than a positive culture of *S typhi*.

Antigen detection as an alternative to Widal agglutination

While bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. In an acute febrile illness in an endemic typhoid region where the clinical picture is ambiguous, a rapid, accurate, specific and sensitive test should be used to differentiate typhoidal from non-typhoidal febrile illnesses. Clinicians usually elect to treat, rather than wait for blood or stool culture results, which may take 3–5 days. While there might be some merit in this approach, particularly in areas where culture facilities are either poor or not available, and where Widal testing is the norm, the use of rapid antigen screening directly from the stool of the suspected patient would be more useful. Khan *et al* have described a new rapid immuno-enzymatic dipstick test for detection of *Salmonella* directly from the stool. The test which is non-invasive, involves homogenisation of stool sample in a buffer solution and immersion of a dipstick (previously coated with antibodies) in a tube containing the supernatant from the homogenised stool samples. The contents of the tube (dipstick and supernatant) are incubated at room temperature for 15 min and a second tube is incubated for an additional 5 min for full development of colour. The dipstick is air dried and the result is visualised as a horizontal mark on the dipstick. While this test is new, Khan *et al* have reported a preliminary sensitivity of 94%, specificity of 98%, negative predictive value of 99.5%, and positive predictive value of 74%. A large-scale field trial is underway to determine the true sensitivity, specificity and the predictive values. It is hoped that such a direct stool testing will be a useful discriminating test which can be used with confidence in areas where both malaria and typhoid may have similar clinical presentations.

Conclusion

More than 100 years after the introduction of the Widal test for diagnosis of typhoid fever, the controversy that surrounded the test has not abated. It has become increasingly obvious that bacterial agglutination systems (particularly Widal), while offering a simple methodology, often result in misleading information because of the polyvalent nature of the antigens involved. Whereas cross-reacting antigens are widely distributed in the microbial world, the specificity and sensitivity of bacterial agglutination is not sufficient when used in human serum assays. We believe that Widal test cannot be expected to give a reliable diagnostic result in endemic regions for the following reasons: the inherent variabilities of the test, difficulty in establishing a steady-state baseline titre for the population, repeated exposures to *S. typhi* in endemic regions, cross-reactivities with other non-*Salmonella* organisms, lack of reproducibility of the test result. The use of Widal agglutination does have limitations. As cultures are time consuming, increased efforts should be made to find a better, more rapid, sensitive and specific test (such as antigen screening/ or antibody assessment by a rapid device technique) to supplement clinical and culture data.

ENTEROCHECK-WB

Rapid test for the detection of IgM antibodies to *S. typhi*

- Specific IgM antibodies to *S.typhi* can be detected – *S.typhi* 'O' specific LPS (Lipopolysaccharide) antigen used in capture region.
- Suitable for pediatric patients, for whom sample collection is a critical issue – only 5µl sample is required. Even finger prick sample can be used.
- No paired sera testing required – Detects only IgM class of antibodies. Sensitive detection than widal & other rapid test – LPS antigen used in test system.
- Sensitive detection capacity – LPS antigen used in test system. (IgM antibodies develop first against LPS antigen in typhoid seroresponse)
- Reliable results – 100% sensitivity and 97.7% specificity.

Detects the CASE, despite the BASE.

BOUQUET

In Lighter Vein

Mr. and Mrs. Brown had two sons. One was named Mind Your Own Business & the other was named Trouble. One day the two boys decided to play hide and seek. Trouble hid while Mind Your Own Business counted to one hundred. Mind Your Own Business began looking for his brother behind garbage cans and bushes. Then he started looking in and under cars until a police man approached him and asked, "What are you doing?" "Playing a game," the boy replied. "What is your name?" the officer questioned. "Mind Your Own Business." Furious the policeman inquired, "Are you looking for trouble?!" The boy replied, "Why, yes."

Little Susie, a six-year-old, complained, "Mother, I've got a stomach ache." "That's because your stomach is empty," the mother replied. "You would feel better if you had something in it." That afternoon, her father came complaining that he had a severe headache all day. Susie perked up, "That's because it's empty," she said. "You'd feel better if you had something in it."

Wife: "In my dream, I saw you in a jewelry store and you bought me a diamond ring."
Husband: "I had the same dream and I saw your dad paying the bill."

A wife comes home late one night and quietly opens the door to her bedroom. From under the blanket, she sees four legs instead of just her husband's two. She reaches for a baseball bat and starts hitting the blanket as hard as she can. Once she's done, she goes to the kitchen to have a drink. As she enters, she sees her husband there, reading a magazine. He says, "Hi darling, your parents have come to visit us, so let them stay in our bedroom. Did you say hello?"

There is a senior citizen driving on the highway. His wife calls him on his cell phone and in a worried voice says, "Herman, be careful! I just heard on the radio that there is a madman driving the wrong way on Route 280!" Herman says, "I know, but there isn't just one, there are hundreds!"

A 3 years old boy sits near a pregnant woman.
Boy: Why do you look so fat?
Pregnant woman: I have a baby inside me.
Boy: Is it a good baby?
Pregnant woman: Yes, it is a very good baby.
Boy: Then why did you eat it?

Wisdom Whispers

To be rich,

is not what you have in your bank account, but what you have in your heart.

Some people won't like you because your strength reminds them of their weakness. Don't let their negativity slow you down.

Soul Quest

PRAYER is when you talk to GOD, MEDITATION is when GOD talks to you.

GOD ANSWERS IN 3 WAYS:
He says yes and gives you what you want.
He says no and gives you something better.
He says wait and gives you the best.

Brain Teasers

1. **Which test is usually first recommended for an infertile couple?**
   A. Seminogram (semen analysis) B. Ultrasound
   C. Endometrial biopsy D. None of the above.

2. **Spermatozoa (derived from testis) comprise about of semen volume.**
   A. 2% B. 5%
   C. 10% D. 20%.

3. **Approximately % of the semen volume is derived from the seminal vesicles.**
   A. 20 B. 40
   C. 60 D. 80.

4. **The color of semen is because of:**
   A. Bilirubin B. Hemoglobin
   C. Cytochromes D. Flavin.

ANSWERS: 1.A, 2.B, 3.C, 4.D.

TROUBLESHOOTING

PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions.

Observation	Possible Cause	Solution
SEQUENCE ERRORS	Low fidelity polymerase	● Choose a higher fidelity polymerase DNA Polymerases
	Suboptimal reaction conditions	● Reduce number of cycles ● Decrease extension time ● Decrease Mg^{++} concentration in the reaction
	Unbalanced nucleotide concentrations	● Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	● Start with a fresh template ● Try repairing DNA template ● Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	● Clone into a non-expression vector ● Use a low-copy number cloning vector
	Incorrect annealing temperature	● Recalculate primer
INCORRECT PRODUCT SIZE	Mispriming	● Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{++} concentration	● Adjust Mg^{++} concentration in 0.2–1 mM increments
	Nuclease contamination	● Repeat reactions using fresh solutions
	Incorrect annealing temperature	● Recalculate primer ● Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
NO PRODUCT	Poor primer design	● Check specific product literature for recommended primer design ● Verify that primers are non-complementary, both internally and to each other ● Increase length of primer
	Poor primer specificity	● Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	● Primer concentration can range from 0.05–1μM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	● Repeat reaction setup
	Suboptimal reaction conditions	● Optimize Mg^{++} concentration by testing 0.2–1 mM increments ● Thoroughly mix Mg^{++} solution and buffer prior to adding to the reaction ● Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair

Observation	Possible Cause	Solution
NO PRODUCT	Poor template quality	● Analyze DNA via gel electrophoresis before and after incubation with Mg^{++} ● Check 260/280 ratio of DNA template
	Presence of inhibitor in reaction	● Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit ● Decrease sample volume
	Insufficient number of cycles	● Rerun the reaction with more cycles
	Incorrect thermocycler programming	● Check program, verify times and temperatures
	Inconsistent block temperature	● Test calibration of heating block
	Contamination of reaction tubes or solutions	● Autoclave empty reaction tubes prior to use to eliminate biological inhibitors ● Prepare fresh solutions or use new reagents and new tubes
	Complex template	● Use Q5 High-Fidelity ● For GC-rich templates
	Premature replication	● Use a hot start polymerase ● Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
MULTIPLE OR NON-SPECIFIC PRODUCTS	Primer annealing temperature too low	● Increase annealing temperature
	Incorrect Mg^{++} concentration	● Adjust Mg^{++} in 0.2–1 mM increments
	Poor primer design	● Check specific product literature for recommended primer design ● Verify that primers are non-complementary, both internally and to each other ● Increase length of primer ● Avoid GC-rich 3´ ends
	Excess primer	● Primer concentration can range from 0.05–1µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	● Use positive displacement pipettes or non-aerosol tips ● Set-up dedicated work area and pipettor for reaction setup ● Wear gloves during reaction setup
	Incorrect template concentration	● For low complexity templates (i.e. plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction ● For higher complexity templates (i.e. genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

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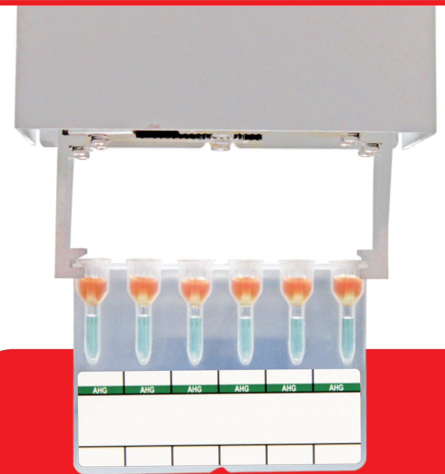

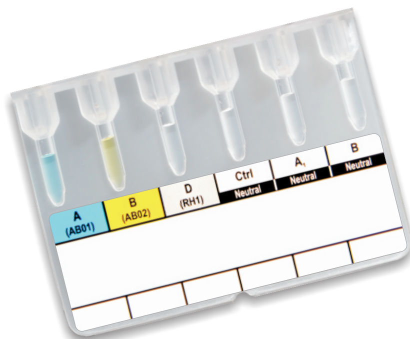

Automation → because Patients safety is our priority...

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