Testing for antibody in blood: how useful?

Testing for antibody in blood continues to be a useful and essential means of safeguarding public health by ensuring a safe blood supply, determining immune status in vaccinated or previously infected individuals, diagnosis of infection, determination of disease stage and disease monitoring.

The advent of more sensitive virologic tests for the detection and monitoring of viremia has helped close the gaps in sensitivity that exist with current serologic tests. However, virologic testing cannot supplant serologic testing due to the immune system's ability to clear viremia in a short period of time relative to the display of protective humoral and cellular immunity in immunocompetent individuals. Virologic and serologic testing therefore work synergistically and complementarily, in the hands of laboratorians and physicians, to protect the blood supply and manage the course of disease.

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Volunteer blood donor sera in Australia are screened by the Australian Red Cross Blood Service for the following infectious agents using antibody tests: Syphilis, Hepatitis B and C, HIV, and HTLV [1]. Testing for HIV and HCV includes both antibody and nucleic acid testing in order to detect infection at an earlier stage as viremia precedes the appearance of antibody present in donor blood during the acute stage of HIV and HCV infection.

In a recent study [2] performed in the United States, mini-pool nucleic acid testing has helped prevent the transmission of five HIV-1 and 56 HCV infections annually by closing the marker negative gap in infectious units by several days. Nevertheless, as these blood donors proceed through infection, RNA levels usually drop below detectable levels, while antibody levels continue to increase.

The diagnosis of disease, disease stage, and disease monitoring has been greatly facilitated through the use of a combination of tests that detect both antigen and antibody in blood. Physicians will usually order an acute viral hepatitis panel when a patient presents clinical symptoms that include elevated bilirubin and liver enzymes.

This panel usually consists of four tests; Hepatitis B surface antigen (HBsAg), Anti-Hepatitis B core IgM (Anti-HBc IgM), Anti-Hepatitis A IgM (Anti-HAV IgM), and Anti-Hepatitis C antibody (Anti-HCV Ab) [3]. If the patient, for example, is positive for HBsAg and Anti-HBc IgM and negative for Anti-HAV IgM and Anti-HCV, the diagnosis of an acute Hepatitis B infection has been

Table 1.   Antigen and antibody testing for monitoring Hepatitis B infection and immunity.

| Patient status | Hepatitis B monitoring panels* | | | | | |
	HBsAg	HBeAg	Anti-HBs Ab	Anti-HBe Ab	Anti-HBc IgM	Anti-HBc IgG
Acute infection (<3 months)	+	+ (early) – (late)	–	– (early) + (late)	+	+
Chronic infection	+	+/–	–	+/–	–	+
Past infection	–	–	+	+	–	+
Hepatitis B vaccine recipient	–	–	+	–	–	–

* HBsAg	*Hepatitis B surface antigen*		Anti-HBe Ab	*Anti-Hepatitis B envelope antibody*
HBeAg	*Hepatitis B envelope antigen*		Anti-HBc IgM	*Anti-Hepatitis B core IgM*
Anti-HBs Ab	*Anti-Hepatitis B surface antibody*		Anti-HBc IgG	*Anti-Hepatitis B core IgG*

established. As shown in Table 1, this patient would then be monitored by the physician through a combination of Hepatitis B surface/envelope/core antigen and antibody blood tests to determine if the patient has resolved the infection and assess the potential for the development of chronic disease [4]. The development of protective immunity in individuals vaccinated with the recombinant Hepatitis B vaccine, which contains only HBsAg, is determined through the use of a quantitative anti-Hepatitis B surface antibody assay.

During the course of viral disease progression, IgM and IgG antibodies are produced with a transient appearance of viremia in immunocompetent individuals. Generally speaking, when viral IgM and IgG antibodies are present in the blood, the patient is considered to be in the acute phase of the infection (primary infection), whereas when only IgG antibodies are present and IgM antibodies and viremia are absent, the patient is considered immune (past infection) (Table 1).

Unfortunately, for Herpes viruses like human cytomegalovirus (HCMV) that establish latency in the host, viral reactivation can occur, resulting in the secondary production of IgM antibodies (non-primary infection) years after primary infection [5]. This obviously complicates the interpretation of HCMV serologic test results on a single blood specimen as the mere presence of both HCMV-specific IgG and IgM does not establish the diagnosis of a primary HCMV infection.

For example, it is important for a physician to determine if the HCMV infection in a pregnant woman is primary or non-primary, as the former presents a

significant risk of foetal damage if intrauterine transmission of the virus occurs during the first trimester. Recent studies indicate that virologic maternal tests are not adequate to diagnose a recent primary maternal infection, and detection or determination of viral load in maternal blood does not correlate with a higher risk for foetal infection [6].

Resolution of this conundrum to some extent has been achieved by the observation that, for a number of viral diseases, the functional binding affinity or avidity of IgG antibodies produced in response to primary viral infection increases with time and can be used to determine the approximate stage of infection [7]. Hence, as shown

schematically in Figure 1, for pregnant women with positive test results for HCMV-specific IgG and IgM in the first trimester, a HCMV avidity IgG test should be performed to determine if the HCMV infection is primary or non-primary. If the HCMV infection is primary, then virologic tests should be performed on amniotic fluid and not on maternal blood in order to establish that actual transmission of the virus has occurred.

In addition to HCMV being the most common congenital infection in newborn infants [5], it is also a leading cause of morbidity and mortality in solid organ transplant recipients [8]. It is a routine practice to determine the HCMV antibody status of both donor and recipient prior

Figure 1. HCMV testing algorithm for first trimester pregnant women.
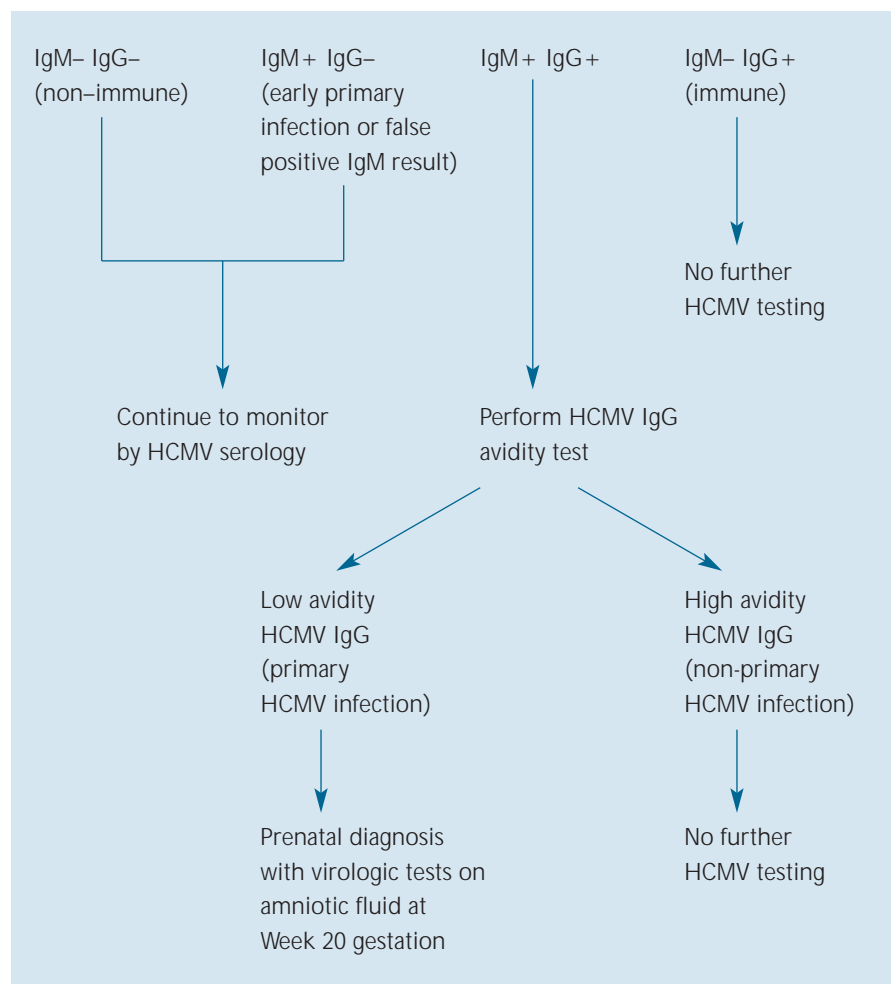
to transplantation in order to assess the risk of HCMV infection in the transplant recipient and determine whether ganciclovir anti-viral prophylaxis should be administered. The transplant recipient is usually followed by HCMV virologic tests to monitor the risk of infection, as these methods are more sensitive than HCMV serologic tests. In addition, the immunosuppressive regimens used to prevent graft rejection suppress the immune system and the concomitant production of antibodies.

Following the development of a new sensitive assay for the detection of HCMV-specific IgM, the Abbott AxSYM CMV IgM assay [9], a retrospective study was performed to re-examine the utility of following liver transplant recipients longitudinally for HCMV infection by PCR and by the AxSYM assay for HCMV-specific IgM [10]. The detection of HCMV-specific IgM post-transplant was significantly associated with the development of HCMV disease in patients who were positive for HCMV DNA.

Furthermore, following liver transplant recipients by both PCR and HCMV, IgM serology was complementary, as two patients, in this 40 patient cohort who progressed to HCMV disease, were identified earlier by HCMV serology than PCR. These two patients had organ involvement with HCMV infection (gastrointestinal and lung).

One of the limitations of detection of HCMV infection in the blood by PCR is the tacit assumption that events in the peripheral blood parallel those occurring in target organs. Although this is generally the case, the immune system can detect organ involvement with HCMV and produce antibodies that can be measured in the peripheral blood regardless of the site of infection.

In summary, the detection of antibodies in blood is useful and often complementary to virologic tests in protecting the blood supply, determining the immune status of individuals, and monitoring the course of infection.

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