Current laboratory diagnosis of hepatitis B virus infection including 8 years of retrospective laboratory data

Hepatitis B is far more infectious than HIV.

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The hepatitis B virus (HBV) is a blood-borne pathogen approximately 75 - 200 times more infectious than the human immunodeficiency virus (HIV), and hyper-endemic in most parts of sub-Saharan Africa.[1]

Most acute HBV infections in adults (~90%) are self limiting, with only a small proportion (<1%) of cases progressing to severe acute or fulminant hepatitis. However, the virus can cause a complex chronic infection which is currently estimated to affect 350 - 400 million people worldwide. Approximately one-quarter[2] of this infectious pool of carriers are at risk of developing chronic active disease, or more severe sequelae such as liver cirrhosis and hepatocellular carcinoma. These complications can be prevented or delayed with therapy, but not all carriers are candidates for therapy, and a precise diagnosis is important for proper patient management.

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Natural history of HBV infection

The small envelope protein, HBsAg, is the first viral marker to appear after HBV infection, and may be found before symptoms or abnormalities in blood chemistry (Fig. 1).[3] The soluble e antigen, HBeAg, becomes detectable shortly thereafter. The first indirect marker, IgM antibody to the nucleocapsid or core antigen (HBcIM), develops approximately 2 weeks after the appearance of HBsAg, and disappears some 24 weeks later, being gradually replaced by IgG antibodies to the core (Fig. 1). The time to HBeAg seroconversion may be short (a few weeks) or greatly delayed (10 - 30 years). Persistence of this phase for >20 weeks has been shown to increase the risk of progression to chronic HBV infection. The latter is defined by persistence of HBsAg and failure to develop neutralising surface antibodies (HBsAb) more than 6 months after infection (Fig. 2).[3]

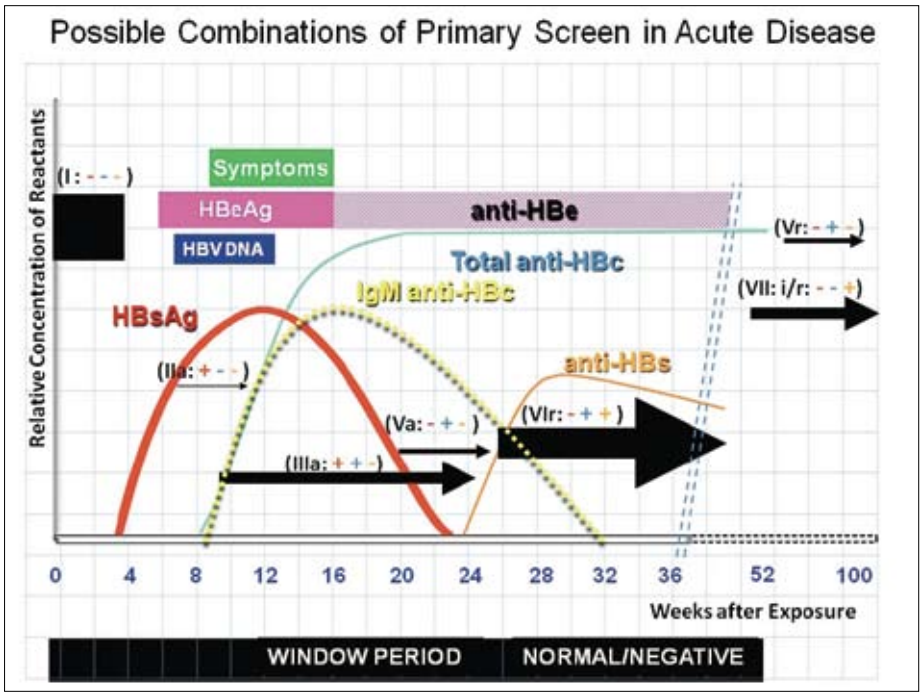

Fig. 1. Possible combinations of primary screen in acute disease. Six of the possible primary marker combinations are shown. They are numbered I - VII according to their first appearance during the timeline of infection and the arrows below them are scaled according to the prevalence of the combination in the laboratory dataset. Marker combination IV (+ + +) occurs when HBsAb develops before HBsAg is undetectable (not shown). (a = acute; r = resolved infection; i = immunity.)

Chronic HBV infection

Five to ten per cent of acutely infected adults, 90% of neonatally infected infants and 25 - 30% of infected children become persistently infected with HBV and develop chronic liver disease of varying severity. Ultimately the dominant cause of viral persistence is a weak antiviral immune response to the HBV antigens, and this has been shown to depend on the specific viral isolate, the sex and human leucocyte antigen (HLA) background of the host, age at time of infection and infectious dose of the virus.[4]

The variability of chronic hepatitis disease can be divided into 4 distinct phases defined by alanine (ALT) and aspartate (AST) transaminase elevations, time of HBeAg seroconversion, HBV DNA levels and concomitant liver damage (Fig. 3). However, not all chronic patients experience all phases of persistent infection and patients can also move from an immune active to an inactive phase and vice versa.[5]

The earliest phase of chronic infection – the immune tolerant phase – is characterised by very high viral loads together with an absence of symptoms, normal ALT levels and minimal liver damage. Tolerance is important in neonatal infection and is most typical in perinatally infected children, adolescents and young adults due to the immaturity of their immune systems.[5]

As the immune system matures, the immunosuppressive effects of tolerance are replaced by an active immune response. The latter combines elements of both innate and adaptive immunity as the host attempts to ward off the foreign pathogen, and the carrier enters the immune clearance phase of HBV chronic disease.[6] Most infected adults progress to this phase of persistence without first experiencing immune tolerance. This phase is characterised by elevated ALT levels, as infected hepatocytes are recognised and destroyed and levels of viral DNA and HBeAg fluctuate. This active immune phase normally transitions spontaneously, or by immune suppression, to a more quiescent, non-replicative phase, the immune control phase, with seroconversion to HBeAb, but it should be noted that this transition can also be reversed.

In up to one-third of patients these flares of disease activity occur without the reappearance of HBeAg. HBeAg-negative chronic hepatitis is often more severe than HBeAg-positive disease and is a consequence of mutant viruses incapable of producing normal levels of the e antigen that now manage to evade the immune system. Therefore, this phase is sometimes called the immune escape phase although it also represents a phase of immune clearance (Fig. 3).[5]

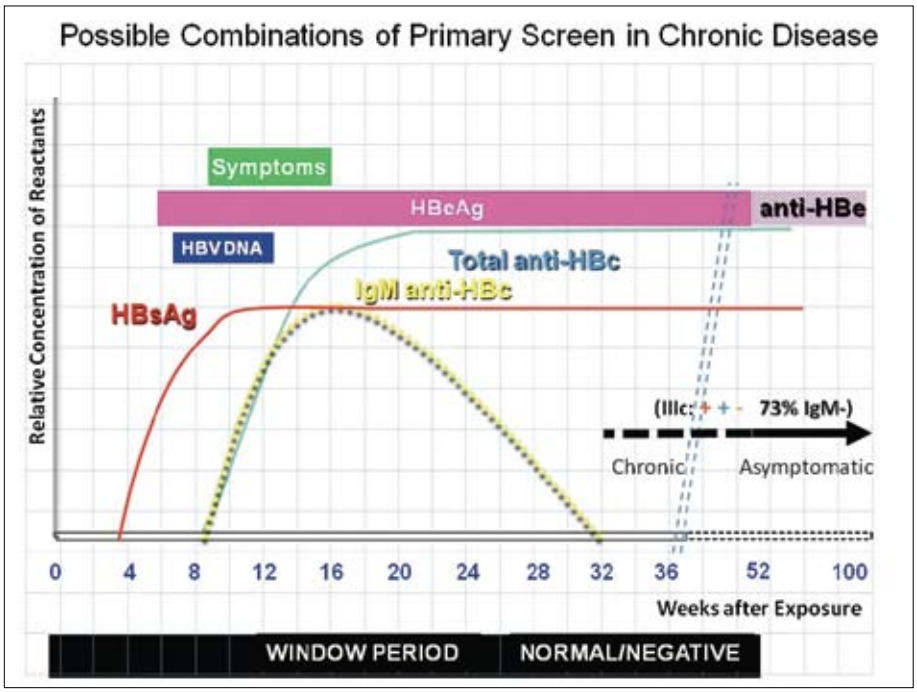

Fig. 2. Possible combinations of primary screen in chronic disease. Marker pattern III (+ + -) can also be indicative of chronic disease (c = chronic) which is defined by persistence of HBsAg carriage for greater than 6 months.

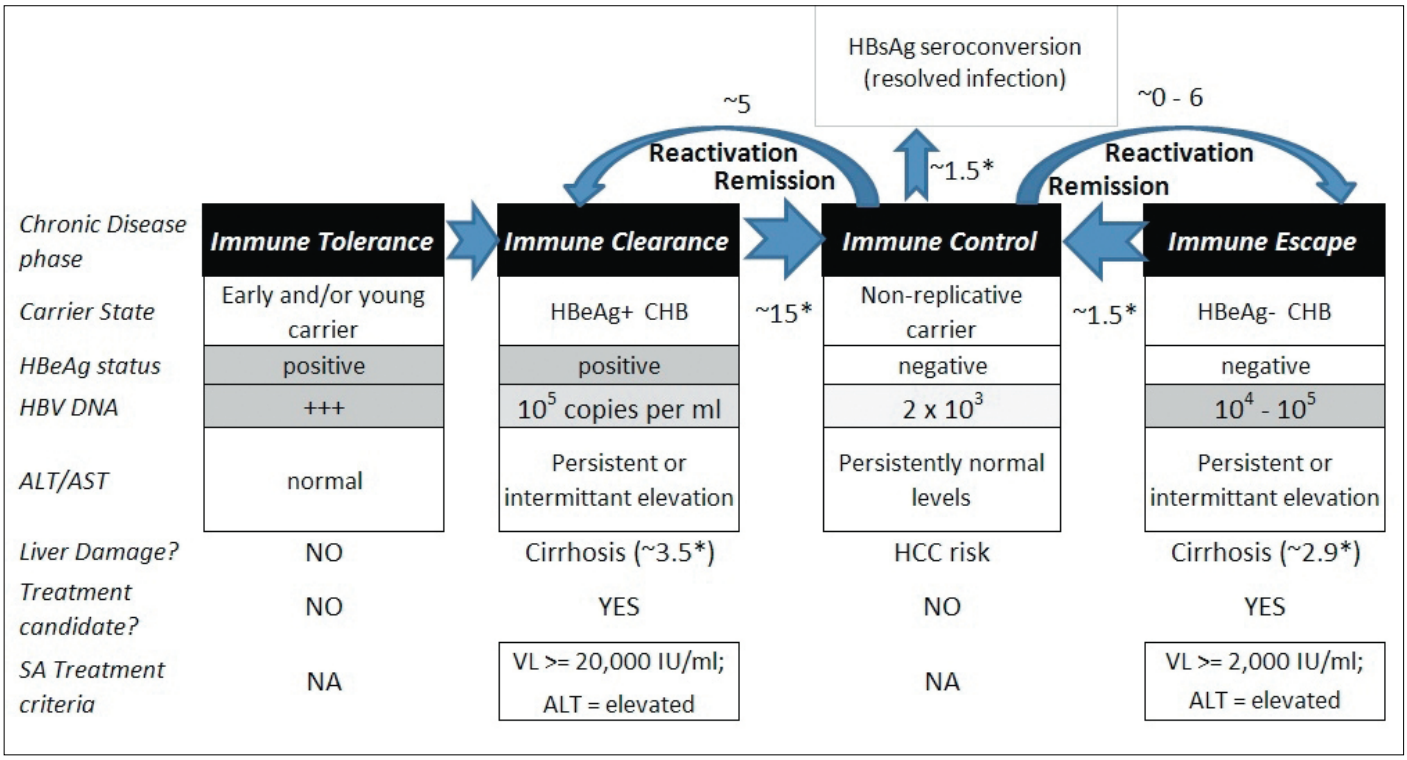

Fig. 3. The phases of HBV chronic carriage. The phases of disease are shown from left to right and include: immune tolerance, immune clearance, immune control and immune escape. The phases are defined according to levels of HBeAg, HBV DNA, AST/ALT and liver damage. Figures followed by an asterisk refer to the 'percentage per year' as opposed to the absolute percentages and were obtained from www.epgonline.org and ref. 14. Recommended treatment criteria for South African chronic hepatitis B (CHB) patients are given. (ALT = alanine transaminase; AST = aspartate transaminase; HCC = hepatocellular carcinoma; VL = hepatitis B virus viral load; IU/ml = international units per millilitre; ~5 copies per millilitre = 1IU.)

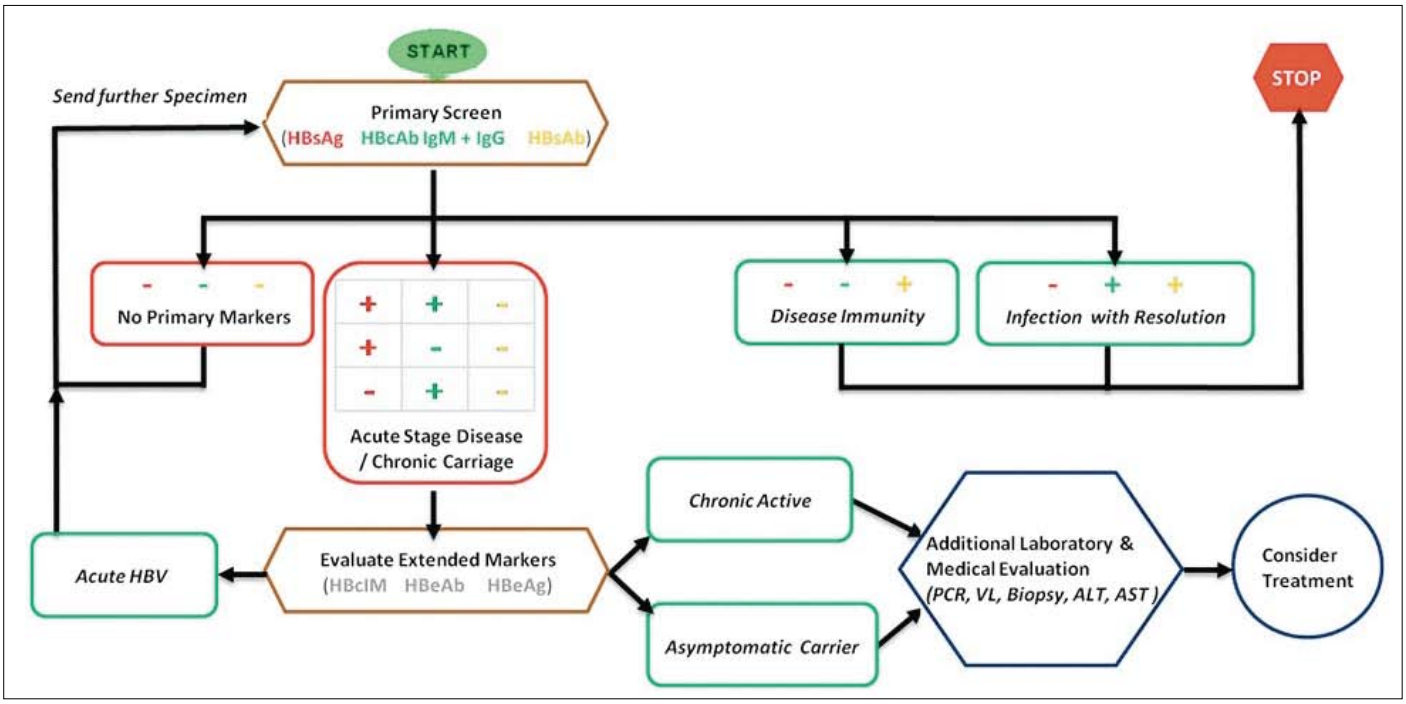

Fig. 4. Flow diagram depicting the steps to be followed in diagnosis of HBV disease. The algorithm is based on a primary and secondary (or extended) screen of serological markers. (PCR = polymerase chain reaction; VL = viral load; ALT = alanine transaminase; AST = aspartate transaminase.)

Table I. Frequency of 8 possible primary screen combinations (*N*=39 774). Seven of the 8 primary marker combinations are assigned to a stage of disease. Combination III and IV are combined to form a single group (see text) and the rarely seen 8th combination (HBsAg and HBsAb together without HBcAb) was not assigned (NA) to a stage of disease.

Stage	HBsAg	Total HBcAb	HBsAb	Percentage	Interpretation
I	-	-	-	52.6	Negative
II*	+	-	-	0.3	Negative or very early disease
III*	+	+	-	8.9	Acute or chronic disease
IV	+	+	+	1.2	Process with pattern III
V*	-	+	-	4	1. Resolving acute window period 2. Resolved infection 3. Passive transfer of HBcAb 4. Occult infection
VI	-	+	+	24.1	Resolved infection
VII	-	-	+	8.9	Immune (most probably vaccination)
NA	+	-	+	0.03	

*Serology patterns which require further testing for secondary markers.

Laboratory diagnosis of HBV infection

Eight years of laboratory data

A baseline, retrospective study by pathologist John Sim[7] examined 39 774 HBV serology records generated by a public sector diagnostic laboratory over 8 years (1985 - 1992). These records represented 35.8% of routine specimens received by the laboratory, which mainly serves specialist referral clinics where patients with known liver disease are studied. All specimens had been subjected to a full primary screen for HBV disease status, which consisted of HBV surface antigen (HBsAg), total core antibody (HBcAb IgM and IgG) and antibody to surface antigen (HBsAb). The aim of the study was to improve interpretive comment generation for routine hepatitis laboratory reporting by identifying all possible patterns of these primary markers. Representative specimens were investigated further by studying the distribution of extended marker frequencies within the groups defined by the primary marker patterns. Extended markers consisted of the e antigen (HBeAg) and antibody (HBeAb) and IgM antibody to the core (HBcIM). Patterns were reconciled with standard profiles of HBV serology (Figs 1 and 2) and then related to possible stages of infection and disease severity.

The study period encompasses the beginning of the exponential phase of the HIV epidemic in South Africa, which is reported to have begun in 1987, reaching equal prevalence in the homo- and heterosexual communities in 1991[8] and rising from a prevalence of 0.7 - 2.2% in pregnant women in the 3 years from 1990 to 1992.[9] According to the National HIV and Syphilis Antenatal Sero-prevalence Survey, the percentage of pregnant women infected with HIV peaked in 2005 and has been stable over the last 4 years. From these records, it is estimated that in 2009 17.8% of the South African adult population (aged 15 - 49 years), or 5.63 million adults and children, were infected with HIV and AIDS.

The specimens partitioned into 8 ($N=2^3$) primary marker patterns or combinations of HBsAg, total HBcAb and HBsAb, and their observed prevalence in the dataset, are shown in Table I. As anticipated, some unusual patterns of primary markers were detected. As busy diagnostic laboratories need to interpret all marker combinations, 7 of the 8 groups were included in the breakdown of extended marker results (HBeAg, HBeAb and HBcIM). Only the extremely rare combination of HBsAg and HBsAb together, without HBcAb, which occurred in 12 of the 39 774 specimens (a frequency of 0.03%), was not assigned to a stage of disease (Table I).

The remaining 7 marker combinations, labelled I - VII according to their first possible occurrence on the timeline of disease progression (Table I and Figs 1 and 2), are discussed below.

I. Negative primary screen, '- - -'
Almost 53% (52.6%) of specimens were negative for all the primary markers and required no further testing.

II. Negative or very early disease, '+ - -'
Similarly, isolated HBsAg (present in only 0.3% of specimens) is most likely nonspecific, particularly if the antigen is detected at low levels. However, since this pattern could represent early stage disease, extended markers should be screened. If these too are found to be negative (as they were 83.2% of the time), the comment returned would be 'negative or early disease'.

The appropriate laboratory report for this, and the previous 'negative' pattern, should, however, advise sending of a further specimen if clinically indicated.

III. Acute or chronic disease, '+ + -'
The presence of surface antigen with total core could indicate acute disease (prior to loss of HBsAg and the development of HBsAb), but is more typical of chronic carriage where HBsAg persists and HBsAb does not develop. This pattern can be better interpreted using the secondary markers.

The presence or absence of HBcIM indicates either acute or early chronic infection, respectively. Chronicity is confirmed if HBsAg persists for >6 months. In either case, the presence of HBeAb indicates a low level of viral replication with low to moderate infectivity, while HBeAg indicates high viral replication and infectivity.

Interestingly, with the exception of isolated HBeAb (which was found to be significantly more prevalent in patients with seropattern III), the distribution of both primary and secondary markers was not significantly different in the specimens with primary seropattern III and IV ('+ + +'), where all 3 primary markers are detected together (Table I). Based on this uncanny marker distribution, it was reasoned that the patients with marker combination IV formed a subset of the patient group with pattern III, and the groups were collapsed into one for diagnostic purposes. Temporally, patients in group IV are acute patients in the early stage of convalescence who are beginning to seroconvert to HBsAb while their HBsAg is still detectable.

Taken together, these patterns were found in just over 10% of the dataset (8.9% and 1.2% for combinations III and IV, respectively).

V. Isolated total core antibody, '- + -'
Four per cent of the dataset had the 'core antibody only' pattern. Almost 12% (11.6%) of specimens with this combination were also positive for HBcIM, and are most probably representative of the core 'resolving acute' window period when HBsAg is clearing but HBsAb is not yet detectable. Alternate explanations for this serological pattern[10] include resolved infection with loss of HBsAb and passive transfer of HBcAb. Importantly, this rare marker combination is being found more frequently in immunocompromised patients, and has also been associated with low-level infection in the absence of HBsAg, so-called occult infection, which can occur with or without immunosuppression.[11] Although the risk of liver disease in the presence of occult infection has not been adequately studied, the majority of black patients with hepatocellular carcinoma (HCC) and serological evidence of past, but not present, HBV infection, were found to have low-level occult infection.[12]

VI. Resolved infection, '- + +'
Core and surface antibody together is typical of resolved infection and there is no need for further testing. Twenty-four per cent (24.1%), or slightly more than half of the specimens with one or more HBV marker, had this pattern.

VII. Immunity, '- - +'
Isolated surface antibody is typical of immunity, and can represent resolved infection with loss of HBcAb, but is more typically the outcome of vaccination. Either way, no further testing is required. This pattern was found in 8.9% of the dataset.

Confounders

Co-infections can confound serological interpretations, e.g. co-infection with other genotypes or mutants of HBV can result in atypical scenarios, including the presence of HBsAg and HBsAb together as their antibodies may not be cross protective. Co-infection with HIV can change the prevalence of certain marker combinations, and initially worsen disease outcome as immunosuppression results in higher viral load with concomitant increase in risk of transmission.[13] Co- and super-infection with HCV or the delta antigen is also associated with more severe disease progression.[5]

Final comments

As methods of HBV DNA detection become increasingly sensitive, both quantitative and qualitative HBV DNA determination is becoming an important aid in the confirmation of diagnosis and the management of chronic disease. While serology is the primary means of diagnosis in HBV infection, nucleic acid testing aids in correct interpretation of the phase of chronic infection and the patient's candidacy for treatment (Fig. 3). The Guidelines for the Control and Treatment of HBV infection in South Africa (in preparation) recommend medical evaluation and subsequent treatment of persistent disease at tertiary health care facilities (Fig. 4). As an inactive carrier may reactivate to an active state and vice versa, lifelong monitoring of viral load and ALT/AST levels is recommended in both treated and untreated patients.

It should be noted that treatment criteria in the South African guidelines are based on the guidelines of the American Association for the Study of Liver Disease (AASLD)[14] even though there are important differences in black South African patients and those in other hyperendemic parts of the world. For example, only 5% of the former are still HBeAg positive by the time they reach adulthood compared with rates of 40% and higher elsewhere.[15] HCC is the commonest tumour in South Africa, with an annual fatality ratio of 0.97. Ninety per cent of HCC is related to HBV infection. Yet, little is known of viral load cut-off levels in black carriers and treatments effective against replicating HBV DNA may not be suitable for the majority of our carriers at risk for fatal HCC.

References available at www.cmej.org.za

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- The hepatitis B virus (HBV) is hyperendemic in South Africa.
- In 90% of infected adults HBV causes an acute self-limiting disease which rarely (<1%) progresses to severe acute or fulminant hepatitis, but 90% of neonatally infected infants and 25 - 30% of children progress to complex chronic infection.
- The three primary markers of HBV infection are:
  - the surface antigen (HBsAg), which indicates current disease
  - total core antibody (HBcAb IgM and IgG), which indicates present or past infection; and
  - antibody to the surface antigen (HBsAb), which indicates immunity.
- HBsAg-positive specimens are diagnosed further using the following secondary markers:
  - the e antigen (HBeAg), which indicates high viral replication and infectivity
  - the e antibody (HBeAb), which indicates low viral replication and low-to-moderate infectivity
  - the IgM core antibody (HBcIM), which indicates current or recent disease.
- The course of chronic HBV infection can be divided into the following four phases based on markers of replicative or non-replicative disease:
  - the immune tolerant phase
  - the immune clearance phase
  - the immune control phase
  - immune escape.
- Not all chronic patients experience all phases of persistent disease and patients can also move from an immune active to an inactive phase and vice versa.
- The majority of chronic carriers in South Africa acquire asymptomatic HBV disease in the first 5 years of life and have seroconverted to HBeAb by the time they reach puberty.
- Preventive measures (such as better vaccine coverage in infancy) are the best form of control in our population as treatments against replicating HBV DNA may be largely ineffective.