Several important markers are used in the diagnosis and monitoring of hepatitis B infection. The presence of HBsAg is the main indicator that a patient is infected and it is therefore the most suitable marker to be detected in blood banks and in pregnancy screening. Latest pieces of evidence support the role of HBsAg (quantification of HBsAg) as a predictive marker for the anti-HBV treatment response and an accurate differential diagnosis of the stage of infection. The “a” determinant, common among all subtypes dw, yw, dr e yr, is the major immunodominant of HBsAg. The so called “escape mutant”, coming to be problematic in recent years, is a mutant which has undergone substitution, deletion or insertion of amino acid(s) in the main common “a” determinant. Some mutations found in this region, can cause a diminished recognition by specifics antibodies and an emergency in mutant detections. It has been previously reported in the literature that a number of HBsAg mutants demonstrate different levels of reactivity depending on the commercial kit employed and, in particular, that certain mutants may be misclassified as negatives. The most important mutations, described in literature, referred to the following insertions and / or substitution described in Figure 1.

The LIAISON® XL murex HBsAg Quant has been designed by using a fully monoclonal-based assay for the quantitative determination of hepatitis B surface antigen in human serum or plasma samples. Reliable detection of genotypes and comparable sensitivity against mutants is assured by using Mouse Monoclonal Antibodies against highly conserved epitope of the inner region of HBs. The scientific innovation of the product is the use of unique Mouse Monoclonal Antibodies (MoAbs), with balanced reactivity against genotypes and subtypes, which are able to detect I/V inner region if combined with a complex detergents mixture. The same solution of surfactants (denaturation treatment) is used in the immunization process and during the screening of the hybridoma cells for selecting MoAbs with the capability to retain the binding activity under harsh assay conditions. (Figure 2)

A first mixture of monoclonal are used for solid phase while a second mixture with different epitopes are linked with an isoluminol derivative. During the first incubation, HBsAg present in samples binds to the solid phase. During the second incubation, the antibodies for conjugate reacts with HBsAg already bound to the solid phase. After each incubation, the unbound material is removed. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of conjugate, is measured by a photomultiplier as relative light units (RLU) and is directly proportional to HBsAg concentration (referred to NIBSC standard - code 00/588, WHO Second International Standard for HBsAg, subtype adw2, genotype A) present in calibrators, samples and controls.

The LIAISON® XL murex HBsAg Quant assay analytical sensitivity (LoD) is less than or equal to 0.030 IU/mL. The study performed with the Second International Standard for HBsAg, subtype adw2, genotype A, NIBSC code: 00/588, showed a sensitivity of 0.05 IU/mL.

Mouse monoclonal mixture instead of the polyclonal use, as many other assays on the market, is also able to guarantee the best lot to lot consistency against standardization. (Graph.1)