Size : 274 x 218 mm

Qualisa™

Microwell Enzyme Immunoassay | HBsAg

Enzyme Linked Immunosorbent Assay (ELISA) for the detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma
FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

QUALISA™ HBsAg is intended to be used for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma. For professional use .

SUMMARY AND EXPLANATION

QUALISA™ HBsAg is a solid phase Enzyme Linked Immunosorbent Assay (ELISA) that employs highly purified, high affinity Agglutinating sera for HBsAg having reactivity for both *ad* and *ay* subtypes.

PRINCIPLE OF THE ASSAY

Microwell strips are coated with Agglutinating sera for HBsAg. Another Agglutinating sera for HBsAg is conjugated to horseradish peroxidase (HRPO). The sample and the conjugate are added in the coated wells and incubated simultaneously. The wells are washed to remove unbound components. Bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula and absorbances of all the wells are compared with the cutoff value. Any sample having absorbance more than the cutoff value is considered reactive.

KIT COMPONENTS

QUALISA™ HBsAg has following components:

1. Coated microwells : Microwells coated with Agglutinating sera for HBsAg. Ready to use.
   96 Wells : (3x8) x 4 pouches
   192 Wells: (3x8) x 8 pouches
   480 Wells: (3x8) x 20 pouches
2. Positive control: HBsAg positive and HIV / HCV non-reactive serum diluted in stabilizer solution with preservatives.
3. Negative control: Inactivated and stabilized human serum non reactive for HIV-1 and HIV-2, HBsAg and HCV.
4. Conjugate: Agglutinating sera for HBsAg- HRPO conjugate.
5. Conjugate activator: Buffered solution containing activator and preservatives.
6. Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
7. Wash buffer: Buffer containing surfactants (20 X). To be diluted 20 times with distilled or deionized water.
8. Stop solution: Diluted Sulphuric acid. Ready to use.
9. Microwell holder.
10. Instruction for use.
11. ELISA protocol sheet.
12. Plate sealer.

STORAGE AND STABILITY

1. **QUALISA™ HBsAg** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated microwells should be used within one month of opening the pouch. Each pouch contains 3 strips of 8 wells. Any unused well(s) should be resealed with cellotape (and desiccant pouch) to prevent moisture absorption and stored at 2-8°C for future use.
3. In case the desiccant pouch changes colour from blue to white, the strips should not be used.
4. Diluted wash buffer is stable upto one week at 2-8°C.
5. The activated conjugate is stable for 2 weeks at 2-8°C. However, it should preferably be used immediately.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipette.
2. Pipette tips.
3. Incubator.
4. Absorbent sheets.
5. ELISA washer.
6. ELISA reader.
7. Pipetting troughs or boats.
8. Disinfectant.
9. Disposable gloves.
10. Reagent grade water.
11. Timer.
12. Biohazard waste container.
13. Serological pipettes.
14. Clean test tubes for conjugate preparation.

Size : 137 x 218 mm

SAMPLE COLLECTION

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard procedure.
3. Serum or plasma can be used.
4. Specimen should be free of particulate matter and microbial contamination.
5. Preferably use fresh sample. However, specimen can be stored refrigerated for short duration. For long storage, freeze at -20°C or below. Do not freeze samples in frost-free freezer.
6. Specimen should not be frozen and thawed repeatedly.
7. Do not heat inactivate before use.
8. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
9. Sodium azide containing samples should not be used.

PRECAUTIONS

(1). Bring all reagents and specimen to room temperature before use. (2). Do not pipette any material by mouth. (3).Do not eat, drink or smoke in the area where testing is done. (4).Use protective clothing and wear gloves when handling samples. (5). Use absorbent sheet to cover the working area. (6). Immediately clean up any spills with sodium hypochlorite. (7).Dispose off all the reagents and material used as they contain infectious agent. (8).Neutralize acid containing waste before adding hypochlorite. (9).Do not use kit after the expiration date. (10).Do not mix components of one kit with another. (11). Always use new tip for each specimen and reagent. (12). Do not let the dispensing tip of ELISA washer touch liquid in the wells. (13). Do not allow liquid from one well to mix with other wells. (14). Do not let the strips dry in between steps. (15). Incubation times and temperatures other than those specified may give erroneous results. (16). Cross contamination of reagents or samples may give false results. Samples must remain confined to microwells during testing. (17). Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be tapped vigorously and blotted on absorbent paper or towels to minimize residual wash buffer. (18).Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use. (19). Adequate washing of wells is extremely important. Inadequately washed wells will exhibit high background values, and may show false positive values. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross- contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp "snapping" motion of the wrist. Repeat these steps for at least six washes. The wells should then be tapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer. The use of an automated microwell washing system will assure consistent washing of the wells, and is recommended.

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water.)
2. Mix conjugate activator and conjugate as shown in the table below.

Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared from table given below. Mix solution thoroughly before use.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate Activator	10µl	20µl	30µl	40µl	50µl	60µl	70µl	80µl	90µl	100µl	110µl	120µl
Conjugate	500µl	1000µl	1500µl	2000µl	2500µl	3000µl	3500µl	4000µl	4500µl	5000µl	5500µl	6000µl

TEST PROCEDURE

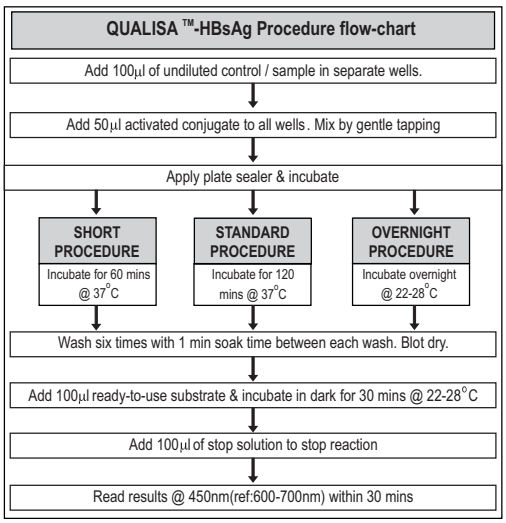
1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare ELISA protocol sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add **100 µl** undiluted control or specimen in separate wells.
6. Add **50 µl** activated conjugate to all the wells. Mix by gentle tapping.
7. Apply plate sealer and incubate according to the procedure chosen.
8. Wash each well by filling **350 µl** diluted wash buffer and aspirating / flicking off six times with 1 minute soak time between each wash. Blot dry.

Procedure	Incubation Time
Short Procedure	60 min. at 37°C
Standard Procedure	120 min. at 37°C
Overnight Procedure	Overnight at 22-28°C

9. Add **100µl** substrate and incubate at 22-28°C away from light for 30 minutes.
10. Stop reaction by adding **100µl** stop solution. The stop solution should be added in the same sequence as substrate addition.
11. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

RUN CRITERIA

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.



CALCULATIONS

The cutoff value is calculated by adding 0.15 to average absorbance value of negative control.

COV = Av.NC + 0.15

Example:

Absorbance of Negative Control (NC)	
Reading -1	0.032
Reading -1	0.040
Average NC reading	0.036

Absorbance of Positive Control (PC)	
Reading -1	2.200
Reading -1	2.150
Average PC reading	2.175

Cutoff value = Av. NC + 0.15
= 0.036 + 0.15
= 0.186

SAMPLE DATA

Well	Absorbance	Mean	Cutoff	Result
NC	0.032	0.036	0.186	
NC	0.040			
PC	2.200	2.175		
PC	2.150			
Sample 1	3.539			Reactive
Sample 2	0.685			Reactive
Sample 3	0.037			Non-Reactive

INTERPRETATION OF RESULTS

1. Samples with absorbance value less than the cutoff value are considered non-reactive by **QUALISA™ HBsAg** ELISA kit and are considered negative for HBsAg.
2. Samples with absorbance value equal to or greater than cutoff value are considered reactive by **QUALISA™ HBsAg** ELISA kit. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicate are considered negative for HBsAg. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
3. If a sample is repeatedly reactive the probability of presence of HBsAg are high, especially with patients at high risk or high absorbance values. Such samples should be retested with confirmatory tests.
4. In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution. This will not interfere with the interpretation of results.

NOTE

All ELISA readers have a linear measuring range (approx. 2.5Å). Beyond this range the OD values are non-linear. That is why some instruments can be pre-programmed to show "out of range" values if the OD exceeds 2.5 or 3.00. **QUALISA™ HBsAg** positive control may show "out of range" values in such instruments even after dilution. Please note that this indicate a valid run (provided the negative control value is < 0.1) and does not interfere with assay results.

PERFORMANCE CHARACTERISTICS

Three hundred forty five specimens – out of which two hundred ten positive and one hundred and thirty-five negative specimens were tested with **QUALISA™ HBsAg** and compared with a commercially available ELISA kit utilizing similar principle. The results are given below:

SPECIMEN DATA	TOTAL	QUALISA™HBsAg	Commercially available ELISA
Number of specimen tested	345	345	345
Number of Positive specimens	210	210	210
Number of Negative specimens	135	135	135

Based on this evaluation:
Sensitivity of **QUALISA™ HBsAg** : 100%
Specificity of **QUALISA™ HBsAg** : 100%

ANALYTICAL SENSITIVITY

Various concentrations of calibrated HBsAg standard were tested and sensitivity of **QUALISA™ HBsAg** in different procedures was found to be as follows.

Procedure	Incubation Time	Sensitivity
Short Procedure	60 min. at 37°C	0.1 ng/ml
Standard Procedure	120 min. at 37°C	0.05 ng/ml
Overnight Procedure	Overnight at 22-28°C	0.025 ng/ml

Precision

Inter & Intra-assay precision studies indicate that the CV is 10 ± 3%.

REMARKS

(1).Though **QUALISA™ HBsAg** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HBV infection. Reactive sample should be retested with confirmatory assays like Neutralization assays, HBV DNA by PCR etc. (2). Absence of HBsAg does not indicate that an individual is absolutely free of HBV infection. (3).Since various tests for HBV differ in their performance characteristics and antibody composition, their reactivity Patterns may differ. (4). Testing of pooled samples is not recommended. (5). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.(6). **QUALISA™ HBsAg** should only be used as a screening test and its results should be confirmed by other supplemental method before taking clinical decisions. (7). Interferences due to heterophile antibodies, Rheumatoid Factors and other nonanalyte substances in patient's serum, capable of binding antibodies multivalently and providing erroneous analyte detection in immunoassays, has been reported in various studies. Though **QUALISA™ HBsAg** uses sufficient amounts of HETEROPHILE BLOCKING REAGENT (HBR) to inhibit the majority of this interference; nevertheless, some samples with high titers may still express clinically important assay interference. Both laboratory professionals and clinicians must be vigilant to this possibility of antibody interference. Results that appear to be internally inconsistent or incompatible with the clinical presentation should invoke suspicion of the presence of an endogenous artifact and lead to appropriate in vitro investigative action.

BIBLIOGRAPHY

(1). Kim, C. Y., Tillis, J. G. 1973, Purification of Biophysical characterization of Hepatitis A antigen, J. Clin. Invest, 52, May 1973, Pgs. 1176-1186.(2). Kee Myung Lee et.al., Emergence of Vaccine- induced escape mutant of Hepatitis B Virus with Multiple surface gene mutations in a Korean child, J.Korean. Med.Sci., 2001, 16, Pgs 356-361.(3). Koyanagi T et al. Analysis of HBs antigen negative variant of hepatitis B virus: Unique Substitutions, Glu 129 to Asp and Gly 145 to Ala in the surface antigen gene. Med Sci Monit, 2000; 6(6): Pgs1165-1169. (4). Data on File: Qualpro Diagnostics.

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