NOTE CHANGES HIGHLIGHTED

ANTIBODY TO HEPATITIS B SURFACE ANTGEN (MOUSE MONOCLONAL):
PEROXIDASE (HORSE RADISH) CONJUGATE

AUSZYME® MONOCLONAL

NAME AND INTENDED USE
AUSZYME MONOCLONAL IS A QUALITATIVE THIRD GENERATION
ENZYME IMMUNOASSAY FOR THE DETECTION OF HEPATITIS B
SURFACE ANTIGEN (HBsAg) IN HUMAN SERUM, PLASMA OR
CADAVERIC SERUM SPECIMENS.

69-4878/R11
1971. In 1976 and 1977, solid phase “sandwich” enzyme immunoassays for antibodies for the detection of HBsAg has previously been reported. Specimens nonreactive by the AUSZYME Monoclonal tests are considered negative for HBsAg and need not be tested further. All specimens considered reactive initially should be repeated tested in duplicate using the same procedure as that used in the initial test. If neither of the repeat tests are reactive, the specimen should be considered negative for HBsAg. If the specimen is reactive in either of the repeat tests, the sample should be considered repetitively reactive. Repetitively reactive specimens should be tested by the HBsAg Test Kit to confirm the AUSZYME results. Only those specimens which the HBsAg can be neutralized by the confirmatory test procedure may be designated as positive for HBsAg.

**BIOLOGICAL PRINCIPLES OF THE PROCEDURES**

**One Step Assay:** In the AUSZYME Monoclonal enzyme immunoassay procedure, beads coated with mouse monoclonal antibody to Hepatitis B Surface Antigen (Anti-HBs) are incubated with serum, plasma or cadaveric serum and Positive and Negative Controls. Any HBsAg present is bound to the solid phase antibody and simultaneously bound by the Anti-HBs-HPO. Unbound material is then aspirated and the beads washed. See **COLOR DEVELOPMENT.**

**Two Step Assay:** In the AUSZYME Monoclonal enzyme immunoassay procedure, beads coated with mouse monoclonal Antibody to Hepatitis B Surface Antigen (Anti-HBs) are incubated with serum, plasma or cadaveric serum and Positive and Negative Controls. Any HBsAg present is bound to the solid phase antibody. After aspiration of the unbound material and washing of the bead, mouse monoclonal Anti-HBs conjugated with horseradish peroxidase (Anti-HBs-HPO) is allowed to react with the antibody-antigen complex. Any anti-HBs that is not isolated by washing is then aspirated and the beads washed. See **COLOR DEVELOPMENT.**

**COLOR DEVELOPMENT**

Next, o-Phenylenediamine (OPD). Solution containing hydrogen peroxide is added to the bead and, after incubation, a yellow-orange color develops in the presence of HBsAg. The enzyme reaction is stopped by the addition of acid. The absorbance of Control and specimen is determined using a spectrophotometer with OPD colorimetric assay. Absorbance of the specimen is compared to the HBsAg of the specimen tested in duplicate. A higher absorbance of the specimen than the Negative Control results in a greater than the absorbance value of the Negative Control Mean plus a factor that are considered reactive initially for HBsAg.

**REAGENTS**

**AUZYME Monoclonal Diagnostic Kit**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUSZYME Monoclonal Kit</td>
<td>1 kit</td>
</tr>
<tr>
<td>Recalified Human Plasma, nonreactive for HBsAg, HIV-1Ag, and HBV-2Ag, and reactive for HBsAg, HIV-1Ag, anti-HIV-2, anti-HIV-1, and anti-HBc. Anti-Hbs:HRPO</td>
<td>1 Vial (5 mL)</td>
</tr>
<tr>
<td>Recalified Human Plasma, reactive for HBsAg, HIV-1Ag, anti-HCV, anti-HIV-2, anti-HIV-1, and anti-HBc. Preservatives: 0.01% Gentamicin Sulfate, 0.1% Thimerosal. Dye: Bromophenol Blue.</td>
<td>5 Vials (9 mL)</td>
</tr>
<tr>
<td>0.02% Hydrogen Peroxide</td>
<td>1 Bottle (55 mL)</td>
</tr>
<tr>
<td>Modified Tris-phosphate buffer containing 0.02% Hydrogen Peroxide</td>
<td>1 Bottle (25 mL)</td>
</tr>
</tbody>
</table>

**Calculation of the Mean Absorbance**

1. Measure the A492 of the OPD/Acid Solution against distilled or deionized water at 0 TIME and 120 MIN.
2. Calculate the Mean Absorbance at 0 TIME and 120 MIN.

**WARNING AND PRECAUTIONS**

**FOR IN VITRO DIAGNOSTIC USE**

**Safety Precautions**

- **CAUTION:** This product contains human sourced and/or potentially infectious components. Some components sourced from human blood has been used and located to be reactive for HBsAg by FDA licensed tests. Refer to the **REAGENT** section of this package insert. No known method that can be complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the **OSHA** Standard on Bloodborne Pathogens. 
- **R43 May cause sensitization by skin contact.**
- **S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.**
- **S20 Water compatible, may be inactivated by heat.**
- **S24/25/26 May cause sensitization by eye contact.**
- **S46 If swallowed, seek medical advice immediately.**
- **S68 In case of contact with skin, rinse immediately with plenty of water and seek medical advice.**
- **S35 This material and its container must be disposed of in a safe way.**
- **S37 This material and its container must be disposed of in a safe way.**
- **S45 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.**

**REAGENT**

**HBsAg Monoclonal Coated Bead Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal): Peroxidase (Horseradish). Minimum Concentration (Mouse) Monoclonal Conjugate. Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal).**

**PROTEIN PREPARATION**

- **ASPERGILLUS NIGER**
  - **AUSZYME®**
  - **1 N Sulfuric Acid, No. 7212-03 (110 mL)**
  - **6 N Sulfuric Acid, No. 7212-03 (110 mL)**
  - **One Step Two Step Assay.**
  - **Controls and specimens is determined using a spectrophotometer with OPD (o-Phenylenediamine > 2HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.**
  - **2H2O2 · HBsAg · Anti-HBs:HRPO**

**COLOR DEVELOPMENT**

1. Pipette 0.2 µL of each of the following standards against distilled or deionized water into each of the five tubes:
- **0 TIME**
- **120 MIN**

2. Add 1.0 mL of 0.1 N Sulfuric Acid under test to each of the five tubes.

3. Measure the A492 of the OPD/Acid Solution against distilled or deionized water at 0 TIME and 120 MIN.

4. Calculate the Mean Absorbance at 0 TIME and 120 MIN.

5. To be acceptable, acid must exhibit:
- a. an A492 of less than 0.04 at 0 TIME and
- b. differences of less than 0.03 units at the values obtained at 0 TIME and 120 MIN.

**DETERMINATION**

1. Wear gloves when handling specimens or reagents.
2. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
3. Clean and disinfect all spills of specimens or reagents using a suitable disinfectant.18,19
4. Clean and disinfect all spills of specimens or reagents using a sublubricoidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.20,21
5. Decommission and dispose of all, specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.22-21
INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

- Bring OPD Reagents to room temperature (15 to 30°C).  

- Use clean pipettes and metal-free containers (such as plastic ware or acid-washed, distilled/deionized water-rinsed glassware), follow the procedure below:

1. Transfer to a suitable container 5 mL of Diluent for OPD for each OPD tablet to be dissolved.

2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of Diluent for OPD using a nonmetallic forceps or disposable, graduated pipettes or dispenser for measuring Diluent for OPD. DO NOT USE A METAL TONGUE THAT IS NOT INACT.

3. Do not use kit beyond the expiration date.

4. Avoid contact of the OPD Substrate Solution and 1 N Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution or 1 N Sulfuric Acid to come into contact with any metal parts. Prior to use, thoroughly rinse glassware used for OPD Substrate Solution with 1 N Sulfuric Acid using approximately 10% of the container volume. Follow with three washes of distilled water at the same volume.

5. Avoid microbial contamination of reagents when removing aliquots from the reagent vials. Use of disposable pipette tips is recommended.

6. If the decaissent obstructs the flow of beads, remove from bead bottle prior to dispensing beads. Replace decaissant in bottle and tightly cap bottle for storage. Do not store beads with decaissant attached to bottle. Any unused beads remaining in the dispenser should be returned to the original container (see Bead Dispenser inserts).

7. In procedure D, do not use nonmetallic forceps or metal-free containers for the OPD Substrate Solution, can be plastic ware or acid-washed, distilled/deionized water-rinsed glassware. (tolerance is ±5%), and 1 mL (tolerance is ±10%).

- A value of less than 0.4 absorbance units (Procedures A, B, and D) and less than 0.2 absorbance units (Procedure C) for the difference between the Positive Control and Negative Control Means (P-N) may indicate deterioration of the kit or OPD reagents. Such runs must be repeated.

PROCEDURE

- The list of accessories required for the COMMANDER Flexible Pipetting Center (FPC™) and Parallel Processing Center (PPC™) are found in the appropriate Data Sheet. The accessories for the COMMANDER PPC, Quantum II, or spectrophotometer capable of reading 1 cm (tolerance is ±5%), and 1 mL (tolerance is ±10%).

- Micro-Lite™ or Metalmaster™ container for storing OPD Substrate Solution, can be plastic ware or acid-washed, distilled/deionized water-rinsed glassware.

- Disposable protective gloves.

- Membrane Seal Punchure Tool for acid bottles.
AUSZYME Monoclonal TEST PROCEDURE

1. The Negative and Positive Controls should be treated as specimens.

PROCEDURAL NOTES

2. Prior to beginning the assay procedure, bring all reagents to room temperature. Prepare and store reagents at the temperatures and time limits specified in the procedure.

PROCEDURE A, B, AND C

1. AUSZYME MC A, B, C, or D. An operator-edited version may be used if the edited lines are consistent with the assay package insert specifications.

2. Insert tray and select the appropriate assay number for either the AUSZYME Monoclonal Tests and/or specimens are subjected to the same processing and incubation times. This may require maintenance of specific

PROCEDURE D

1. Insert tray and select the appropriate assay number for either

CAUTION: Failure to use the Dynamic Incubator in the manner described in the Dynamic Incubator Operator’s Manual may result in incorrect assay results.

COLOR DEVELOPMENT [QUANTUM II AND SPECTROPHOTOMETER]

1. When transferring beads from wells to assay tubes, align inverted rack of the single bead tray, followed immediately by the first assay tray.

PROCEDURE E

1. Remove air bubbles prior to reading absorbance.

PROCEDURE F

1. When using an alternative instrumentation to deliver Controls and specimens, ensure the instrumentation is compatible with this assay.

PROCEDURE G

1. When using an alternative instrumentation to deliver Controls and specimens, ensure the instrumentation is compatible with this assay.

PROCEDURE H

1. Remove air bubbles prior to reading absorbance.

PROCEDURE I

1. When using an alternative instrumentation to deliver Controls and specimens, ensure the instrumentation is compatible with this assay.

PROCEDURE J

1. Remove air bubbles prior to reading absorbance.

PROCEDURE K

1. Remove air bubbles prior to reading absorbance.

PROCEDURE L

1. Remove air bubbles prior to reading absorbance.

PROCEDURE M

1. Remove air bubbles prior to reading absorbance.

PROCEDURE N

1. Remove air bubbles prior to reading absorbance.

PROCEDURE O

1. Remove air bubbles prior to reading absorbance.

PROCEDURE P

1. Remove air bubbles prior to reading absorbance.

PROCEDURE Q

1. Remove air bubbles prior to reading absorbance.

PROCEDURE R

1. Remove air bubbles prior to reading absorbance.

PROCEDURE S

1. Remove air bubbles prior to reading absorbance.

PROCEDURE T

1. Remove air bubbles prior to reading absorbance.

PROCEDURE U

1. Remove air bubbles prior to reading absorbance.

PROCEDURE V

1. Remove air bubbles prior to reading absorbance.

PROCEDURE W

1. Remove air bubbles prior to reading absorbance.

PROCEDURE X

1. Remove air bubbles prior to reading absorbance.

PROCEDURE Y

1. Remove air bubbles prior to reading absorbance.

PROCEDURE Z

1. Remove air bubbles prior to reading absorbance.
PREPARATION OF WATER TUBE (QUANTUM II AND SPECTROPHOTOMETER)
10. Immediately transfer beads to properly identified assay tubes.
11. Prime dispenser immediately prior to dispensing OPD Substrate Solution.
12. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blank), and then into each tube containing a bead.
13. Cover and incubate at room temperature (15-30°C) for 30 minutes.
14. Add 1 mL of 1 N Sulfuric Acid to each tube. Equilibrate to 4°C.

FIRST INCUBATION
5. At the end of the incubation period, remove and discard cover seal.
6. Pipette 200 µL of electrophoresis gel [red] into each well containing a bead. Be careful not to touch the sides of the well with the pipette tip.
7. Apply new cover seal. Gently tap the tray to assure beads are completely covered with liquid and remove any trapped air bubbles.
8. Incubate at 40°C for 1 hour.
9. At the end of the incubation period, remove and discard cover seal.
10. Immediately transfer beads to properly identified assay tubes.

COLOR DEVELOPMENT (QUANTUM II AND SPECTROPHOTOMETER)
15. Pipette approximately 2 mL of distilled or deionized water into an empty tube.

READING (QUANTUM II AND SPECTROPHOTOMETER)
Note: Reading of the assay must be done within 2 hours after acid preparation of the test.

16. In Mode 0, blank the instrument with the water tube (see the Quantum II Operator’s Manual for running in Mode 0).
17. Determine the absorbance of the substrate blank at 492 nm. The substrate blank must be greater than or equal to 0.200 and less than or equal to 0.040. Stop the assay.
18. Select the mode for processing AUSZYME Monoclonal.
19. Determine the absorbance of Controls and test specimens at 492 nm.

QUALITY CONTROL PROCEDURES
For the run to be valid, the difference between the Means of the Positive Control and Negative Control (P-N) should be 0.030 or greater for Procedures A, B, D, and 0.200 or greater for Procedure C. If not, technique may be suspect and the run must be repeated. If the P-N Value is consistently low, deterioration of reagents may be suspect.

1. Substrate Blank Acceptance Criteria
   a. QUANTUM II users: An assay run is considered valid with respect to the substrate blank if the blank has an absorbance value greater than or equal to 0.020 and less than or equal to 0.040. This determination of assay validity due to substrate blank must be done by the user. The substrate blank value is an indication of the integrity of the OPD Substrate Solution. If the substrate blank absorbance falls outside the acceptable range, the preparation of the substrate blank is in question and the alternate substrate blank may be used. If more than an occasional value falls outside the range Mean ± 2.0 Standard Deviation, all Negative Control values should fall within the range Mean ± 2.0 Standard Deviation. In such cases, all Negative Control values should fall within the range Mean ± 0.010. If one value is outside the acceptable range, discard the value and the run must be repeated.
   b. PPC users: Quality control with respect to the substrate blank is determined automatically by the PPC instrument according to the procedure described in the PPC Operations Manual. In the unlikely event, technique errors in the preparation of the OPD Substrate Solution are suspect and the run must be repeated.

2. Calculation of Negative Control Mean Absorbance (NCA)
   a. Determine the Mean of the Negative Control values.
   b. Individual Negative Control values should be less than or equal to 0.030 and greater than or equal to 0.010. Negative Control values should be greater than or equal to 0.050 times the NCA and less than or equal to 1.5 times the NCA. Where the NCA is below 0.030, the calculation of 0.010 x 1.5 times the Mean may be disregarded.

   Example:
   Negative Control
   Sample No. | Absorbance
   ---|---
   1 | 0.010
   2 | 0.011
   3 | 0.009
   Total | 0.030
   Total Absorbance = 0.010 x 0.010 = 0.004

3. Calculation of the Positive Control Mean Absorbance (PCM)
   a. Determine the Mean of the Positive Control values.
   b. PPC users: Quality control with respect to the substrate blank is determined automatically by the PPC instrument according to the procedure described in the PPC Operations Manual. In the unlikely event, technique errors in the preparation of the OPD Substrate Solution are suspect and the run must be repeated.
   c. In the example, no Positive Control sample is rejected as aberrant and the test should be repeated. If more than an occasional value falls outside the range, technique problems must be corrected.

   Example:
   Positive Control
   Sample No. | Absorbance
   ---|---
   1 | 1.054
   2 | 2.054
   TOTAL | 3.108
   Total Absorbance = 2.054 x 1.027 = 2.104

4. Calculation of the P-N Value
   a. Subtract the NCA from the PCM.
   b. P-N Value = 2.104 - 0.004 = 2.094

   Example:
   PCM = 2.104
   NCA = 0.010
   P-N Value = 2.094
   For the run to be valid, the P-N Value must be 0.400 or greater for Procedures A, B, D, and 0.200 or greater for Procedure C. If not, technique or deterioration of reagents may be suspect and the run must be repeated.
RESULTS

1. Calculation of the Cutoff Value

Determine the Cutoff Value for the following Examples below:

Procedure A, B, and C: Add the factor 0.050 to the NC.

Example:

Procedure A

Cutoff Value 0.100

2. Calculation of the Unknown

Procedure C: Add the factor 0.025 to the NC.

Example:

Procedure C

Cutoff Value 0.075

Sensitivity

All highly sensitive immunoassay systems have a tendency to detect all known subtypes of HBsAg. Sensitivity testing demonstrated that AUSZYME Monoclonal Procedure A was also reactive by AUSZYME II (Procedure A).

Specificity

The percentage of specimens found reactive with Procedures A, B, or C and the proportion of these specimens found to be repeatedly reactive were determined by testing 6,583 serum and plasma samples in a clinical investigation performed at four blood banks and at Abbott Laboratories. The presence of HBsAg in the repeatedly reactive specimens was confirmed by neutralization with human Anti-HBs using the HBsAg Confirmatory Assay. The results of these tests are shown in Table II.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Negative Screen</th>
<th>Reactive Screen</th>
<th>Repeatedly Reactive</th>
<th>Confirmed Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>C</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
</tr>
</tbody>
</table>

In these studies, the one reactive specimen tested only by AUSZYME Monoclonal Procedure A was also reactive by AUSZYME II (Procedure A).

In addition, of 128 HBsAg positive specimens, AUSZYME Monoclonal detected all positive all known subtypes that were also reactive by AUSZYME II.

Sensitivity

The relative sensitivity of AUSZYME Monoclonal Procedures A, B, and C was compared using a 35 member proficiency panel of purified HBsAg (a and ay) prepared at Abbott Laboratories. Each panel member was assayed in triplicate by each of the procedures. AUSZYME Monoclonal Procedure A was reactive to purified HBsAg (a and ay) prepared at Abbott Laboratories. Each panel member was assayed in triplicate by each of the procedures. AUSZYME II.

Specificity

The percentage of specimens found reactive with Procedures A, B, or C and the proportion of these specimens found to be repeatedly reactive were determined by testing 6,583 serum and plasma samples in a clinical investigation performed at four blood banks and at Abbott Laboratories.
**PERFORMANCE CHARACTERISTICS OF CASADIVIC SERUM TESTING**

**Reproducibility**

Inter-assay reproducibility of AUSZYME Monoclonal was assessed using 20 postmortem and 20 normal donor sera. These sera specimens were spiked with human plasma positive for Hepatitis B Surface Antigen (HBsAg) to create low-level reactive specimens. Each of the specimens was tested once on six different days on each of three lots of AUSZYME Monoclonal at one site. For inter-assay reproducibility over all lots, the percent coefficient of variation (%CV) ranged from 5.8% to 10.6% for the low-level reactive postmortem specimens, and from 5.3% to 13.3% for the low-level reactive normal donor specimens.

**Specificity**

Specificity was evaluated using 82 postmortem and 65 normal donor sera. Each of the sera specimens was tested once on each of three lots of AUSZYME Monoclonal. The mean sample to cutoff (S/CO) ratio for 186 postmortem replicates (52 specimens with three reagent lots) was 3.925. The mean S/CO ratio for 195 normal donor replicates (65 specimens with three reagent lots) was 0.255. Results are presented in Table V.

**Sensitivity**

Sensitivity was evaluated using 50 postmortem and 50 normal donor sera that were pre-screened for HBsAg and found to be negative. The 50 speciemes were spiked with human plasma positive for HBsAg to create low-level reactive specimens. Each of the specimens was tested once on six different days on each of three lots of AUSZYME Monoclonal. The mean sample to cutoff (S/CO) for 150 postmortem replicates (50 specimens with three reagent lots) was 2.062 and the mean S/CO ratio for 150 normal donor replicates (50 specimens with three reagent lots) was 0.628. The calculated difference between the postmortem specimens and the normal donor specimens was 0.192 S/CO, which was determined not to be statistically significant by the F-test analysis (p-value = 0.045). Results are presented in Table VI.

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### Table V

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of Specimens</th>
<th>No. of Replicates</th>
<th>Mean S/CO</th>
<th>Nonreactive</th>
<th>Repeat Reaction, Non-Confirming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem</td>
<td>50</td>
<td>150</td>
<td>2.062</td>
<td>195 (99.30%)</td>
<td>0 (0.70%)</td>
</tr>
<tr>
<td>Normal Donor</td>
<td>50</td>
<td>150</td>
<td>0.628</td>
<td>195 (98.39%)</td>
<td>1 (1.61%)</td>
</tr>
</tbody>
</table>

The reproducibility of AUSZYME Monoclonal has an estimated specificity of 95.36% (binomial confidence interval 26 = [95.36%, 99.67%]) for postmortem specimens.

### Table VI

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of Specimens</th>
<th>No. of Replicates</th>
<th>Mean S/CO</th>
<th>Reactive, Initially Nonreactive, Repeat Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem</td>
<td>50</td>
<td>150</td>
<td>2.062</td>
<td>150 (100.0%)</td>
</tr>
<tr>
<td>Normal Donor</td>
<td>50</td>
<td>150</td>
<td>0.628</td>
<td>150 (100.0%)</td>
</tr>
</tbody>
</table>

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**BIBLIOGRAPHY**


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