State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
LBS70424	1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
1450 Waukegan Road
McGraw Park, IL 60085

SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
08/01/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT. NO.	ITEM NUMBER	UNIT PRICE	AMOUNT
				REQUEST FOR QUOTATION OPEN-END BLANKET CONTRACT THE WEST VIRGINIA DIVISION OF PURCHASING IS SOLICITING BIDS FOR THE OFFICE OF LABORATORY SERVICES (OLS) TO PROVIDE REAGENTS TO PERFORM EXAMINATION FOR DETECTION OF HEPATITIS A,B,C AND/OR HIV-1 AND HIV-2 PLUS GROUP O IN SERUM SPECIMEN. SELECTED VENDOR MUST PROVIDE A FULLY AUTOMATED ANALYZER AT NO ADDITIONAL CHARGE FOR USE WITH THE REQUESTED REAGENTS. THIS SYSTEM INCLUDES A COMPUTER, MONITOR AND PRINTER, ETC. WHICH WILL BE RETAINED AND MAINTAINED BY THE VENDOR BUT MUST HAVE THE CAPABILITY OF INTERFACING WITH THE LIMS (LABORATORY INFORMATION MANAGEMENT SYSTEM). PLEASE NOTE THE FOLLOWING ATTACHMENTS: 1. LBS70424 SPECIFICATIONS 2. AFFIDAVIT		
0001	100	EA		475-00-99-001	6.01	601.00
				HEPATITIS A - ~~DIASOIN~~ Diasorin ANTI-HAV IGM #P001925 OR EQUAL		
				OPEN-END CONTRACT TO PROVIDE REAGENTS TO PERFORM EXAMINATION FOR DETECTION OF HEPATITIS A, B, C, AND/OR HIV-1 AND HIV-2 PLUS GROUP O IN SERUM SPECIMEN. SELECTED VENDOR		

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wendy Wrum TELEPHONE 800-456-1157 Ext 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

GENERAL TERMS & CONDITIONS
REQUEST FOR QUOTATION (RFQ) AND REQUEST FOR PROPOSAL (RFP)

1. Awards will be made in the best interest of the State of West Virginia.

2. The State may accept or reject in part, or in whole, any bid.

3. All quotations are governed by the ***West Virginia Code*** and the ***Legislative Rules*** of the Purchasing Division.

4. Prior to any award, the apparent successful vendor must be properly registered with the Purchasing Division and have paid the required $125.00 registration fee.

5. All services performed or goods delivered under State Purchase Orders/Contracts are to be continued for the term of the Purchase Order/Contract, contingent upon funds being appropriated by the Legislature or otherwise being made available. In the event funds are not appropriated or otherwise available for these services or goods, this Purchase Order/Contract becomes void and of no effect after June 30.

6. Payment may only be made after the delivery and acceptance of goods or services.

7. Interest may be paid for late payment in accordance with the ***West Virginia Code.***

8. Vendor preference will be granted upon written request in accordance with the ***West Virginia Code.***

9. The State of West Virginia is exempt from federal and state taxes and will not pay or reimburse such taxes.

10. The Director of Purchasing may cancel any Purchase Order/Contract upon 30 days written notice to the seller.

11. The laws of the State of West Virginia and the ***Legislative Rules*** of the Purchasing Division shall govern all rights and duties under the Contract, including without limitation the validity of this Purchase Order/Contract.

12. Any reference to automatic renewal is hereby deleted. The Contract may be renewed only upon mutual written agreement of the parties.

13. **BANKRUPTCY:** In the event the vendor/contractor files for bankruptcy protection, this contract is automatically null and void, and is terminated without further order.

14. **HIPAA Business Associate Addendum** - The West Viginia State Government HIPAA Business Associate Addendum (BAA), approved by the Attorney General, and available online at the Purchasing Division's web site (**http://www.state.wv.us/admin/purchase/vrc/hipaa.htm**) is hereby made part of the agreement. Provided that, the Agency meets the definition of a Covered Entity (45 CFR §160.103) and will be disclosing Protected Health Information (45 CFR §160.103) to the vendor.

INSTRUCTIONS TO BIDDERS

1. Use the quotation forms provided by the Purchasing Division.

2. **SPECIFICATIONS:** Items offered must be in compliance with the specifications. Any deviation from the specifications must be clearly indicated by the bidder. Alternates offered by the bidder as **EQUAL** to the specifications must be clearly defined. A bidder offering an alternate should attach complete specifications and literature to the bid. The Purchasing Division may waive minor deviations to specifications.

3. Complete all sections of the quotation form.

4. Unit prices shall prevail in cases of discrepancy.

5. All quotations are considered F.O.B. destination unless alternate shipping terms are clearly identified in the quotation.

6. **BID SUBMISSION:** All quotations must be delivered by the bidder to the office listed below prior to the date and time of the bid opening. Failure of the bidder to deliver the quotations on time will result in bid disqualifications.

SIGNED BID TO:

Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
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ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
1450 Waukegan Road
McGraw Park, IL 60085

SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
08/01/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

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	MUST PROVIDE A FULLY AUTOMATED ANALYZER AT NO ADDITIONAL CHARGE FOR USE WITH THE REQUESTED REAGENTS. THIS SYSTEM INCLUDES A COMPUTER, MONITOR AND PRINTER, ETC. WHICH WILL BE RETAINED AND MAINTAINED BY THE VENDOR BUT MUST HAVE THE CAPABILITY OF INTERFACING WITH THE LIMS (LABORATORY INFORMATION MANAGEMENT SYSTEM). NOTE: SEE ATTACHED DETAILED SPECIFICATIONS					
0002	10	EA		475-00-99-001	5.99	59.90
	HEPATITIS B - GENETIC SYSTEMS HBSAG CONFIRMATORY #32594 OR EQUAL - Package Insert Included					
0003	2,000	EA		475-00-99-001	4.30	8,595.00
	HEPATITIS B - GENETIC SYSTEMS HBSAG 3.0 EIA #32591 OR EQUAL					
0004	2,000	EA		475-00-99-001	4.98	9,956.00
	HEPATITIS B - DIASORIN ANTI-HBCORE #P001927 OR EQUAL					

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wesley Wrenn TELEPHONE 800-456-1157 Ext 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
1450 Waukegan Road
McGraw Park, IL 60085

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BPH - LABORATORY SERVICES

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08/01/2006				

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LINE	QUANTITY	UOP	CAT. NO.	ITEM NUMBER	UNIT PRICE	AMOUNT
0005	100	EA		475-00-99-001	5.97	597.00
	HEPATITIS B - DIASORIN ANTI-HBC 1GM #P001928 OR EQUAL					
0006	1,000	EA		475-00-99-001	4.89	4,889.00
	HEPATITIS B - DIASORIN ANTI-BBS OR EQUAL					
0007	2,000	EA		475-00-99-001	8.55	17,095.00
	HEPATITIS C - ORTHO HCV EIA V3.0 #930740 OR EQUAL					

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wesley Wagner TELEPHONE 800-456-1157 Ext 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
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08/01/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT. NO.	ITEM NUMBER	UNIT PRICE	AMOUNT
0008	7,000	EA		475-00-99-001	no bid	-0-

HIV - BIORAD HIV 1/2/0 #32588 OR EQUAL

THE NUMBER OF TESTS REQUESTED ARE FOR BIDDING PURPOSES ONLY, AND THE VENDOR WILL BE REQUIRED TO PROVIDE ONLY THE QUANITITY NEEDED, BE IT MORE OR LESS. TEST KITS AR TO BE SHIPPED AS REQUESTED. REAGENTS ARE TO BE SHIPPED WITHIN THREE (3) DAYS OF RECEIVING AN ORDER.

THE TEST KIT MUST HAVE A MINIMUM SHELF LIFE OF NINETY (90) DAYS OR MORE BEYOND DATE OF RECEIPT.

ALL PRODUCTS AND EQUIPMENT ARE TO BE QUOTED FOB DESTINATION, UNLESS OTHERWISE STATED IN VENDOR'S QUOTATION.

EXHIBIT 3

LIFE OF CONTRACT: THIS CONTRACT BECOMES EFFECTIVE ON OCTOBER 16, 2006 AND EXTENDS FOR A PERIOD OF ONE (1) YEAR OR UNTIL SUCH "REASONABLE TIME" THEREAFTER AS IS NECESSARY TO OBTAIN A NEW CONTRACT OR RENEW THE ORIGINAL CONTRACT. THE "REASONABLE TIME" PERIOD SHALL NOT EXCEED TWELVE (12) MONTHS. DURING THIS "REASONABLE TIME" THE VENDOR MAY TERMINATE THIS CONTRACT FOR ANY REASON UPON GIVING THE DIRECTOR OF PURCHASING 30 DAYS WRITTEN NOTICE.

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wendy Adkins TELEPHONE 800-456-1159 Ext 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186

ADDRESS CHANGES TO BE NOTED ABOVE

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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

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1450 Waukegan Road
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UNLESS SPECIFIC PROVISIONS ARE STIPULATED ELSEWHERE IN THIS CONTRACT DOCUMENT, THE TERMS, CONDITIONS AND PRICING SET HEREIN ARE FIRM FOR THE LIFE OF THE CONTRACT.

RENEWAL: THIS CONTRACT MAY BE RENEWED UPON THE MUTUAL WRITTEN CONSENT OF THE SPENDING UNIT AND VENDOR, SUBMITTED TO THE DIRECTOR OF PURCHASING THIRTY (30) DAYS PRIOR TO THE EXPIRATION DATE. SUCH RENEWAL SHALL BE IN ACCORDANCE WITH THE TERMS AND CONDITIONS OF THE ORIGINAL CONTRACT AND SHALL BE LIMITED TO TWO (2) ONE (1) YEAR PERIODS.

CANCELLATION: THE DIRECTOR OF PURCHASING RESERVES THE RIGHT TO CANCEL THIS CONTRACT IMMEDIATELY UPON WRITTEN NOTICE TO THE VENDOR IF THE COMMODITIES AND/OR SERVICES SUPPLIED ARE OF AN INFERIOR QUALITY OR DO NOT CONFORM TO THE SPECIFICATIONS OF THE BID AND CONTRACT HEREIN.

OPEN MARKET CLAUSE: THE DIRECTOR OF PURCHASING MAY AUTHORIZE A SPENDING UNIT TO PURCHASE ON THE OPEN MARKET, WITHOUT THE FILING OF A REQUISITION OR COST ESTIMATE, ITEMS SPECIFIED ON THIS CONTRACT FOR IMMEDIATE DELIVERY IN EMERGENCIES DUE TO UNFORESEEN CAUSES (INCLUDING BUT NOT LIMITED TO DELAYS IN TRANSPORTATION OR AN UNANTICIPATED INCREASE IN THE VOLUME OF WORK.)

QUANTITIES: QUANTITIES LISTED IN THE REQUISITION ARE APPROXIMATIONS ONLY, BASED ON ESTIMATES SUPPLIED BY THE STATE SPENDING UNIT. IT IS UNDERSTOOD AND AGREED THAT THE CONTRACT SHALL COVER THE QUANTITIES ACTUALLY ORDERED FOR DELIVERY DURING THE TERM OF THE CONTRACT, WHETHER MORE OR LESS THAN THE QUANTITIES SHOWN.

ORDERING PROCEDURE: SPENDING UNIT SHALL ISSUE A

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE (Debby Wrenn) TELEPHONE 800-456-1150 EXT 5139 DATE 9/4/06

TITLE S/B Representative FEIN 36-4095185 ADDRESS CHANGES TO BE NOTED ABOVE

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State of West Virginia
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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

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Scientific Products Distribution
1450 Waukegan Road
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WRITTEN STATE CONTRACT ORDER (FORM NUMBER WV-39) TO THE VENDOR FOR COMMODITIES COVERED BY THIS CONTRACT, EXCEPT WHEN PURCHSES ARE OF A DOLLAR AMOUNT ALLOWABLE TO BE MADE WITH THE WV STATE CREDIT CARD (P-CARD).

BANKRUPTCY: IN THE EVENT THE VENDOR/CONTRACTOR FILES FOR BANKRUPTCY PROTECTION, THIS CONTRACT IS AUTOMATICALLY NULL AND VOID, AND IS TERMINATED WITHOUT FURTHER ORDER.

THE TERMS AND CONDITIONS CONTAINED IN THIS CONTRACT SHALL SUPERSEDE ANY AND ALL SUBSEQUENT TERMS AND CONDITIONS WHICH MAY APPEAR ON ANY ATTACHED PRINTED DOCUMENTS SUCH AS PRICE LISTS, ORDER FORMS, SALES AGREEMENTS OR MAINTENANCE AGREEMENTS, INCLUDING ANY ELECTRONIC MEDIUM SUCH AS CD-ROM.

REV. 04/11/2001

VENDOR PREFERENCE CERTIFICATE

CERTIFICATION AND APPLICATION* IS HEREBY MADE FOR PREFERENCE IN ACCORDANCE WITH WEST VIRGINIA CODE, 5A-3-37 (DOES NOT APPLY TO CONSTRUCTION CONTRACTS).

A. APPLICATION IS MADE FOR 2.5% PREFERENCE FOR THE REASON CHECKED:

( ) BIDDER IS AN INDIVIDUAL RESIDENT VENDOR AND HAS RESIDED CONTINUOUSLY IN WEST VIRGINIA FOR FOUR

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE [signature] TELEPHONE 800-456-1152 EXT 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186

ADDRESS CHANGES TO BE NOTED ABOVE

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State of West Virginia
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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

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(4) YEARS IMMEDIATELY PRECEDING THE DATE OF THIS CERTIFICATION; OR

( ) BIDDER IS A PARTNERSHIP, ASSOCIATION OR CORPORATION RESIDENT VENDOR AND HAS MAINTAINED ITS HEADQUARTERS OR PRINCIPAL PLACE OF BUSINESS CONTINUOUSLY I WEST VIRGINIA FOR FOUR (4) YEARS IMMEDIATELY PRECEDING THE DATE OF THIS CERTIFICATION; OR 80% OF THE OWNERSHIP INTEREST OF BIDDER IS HELD BY ANOTHER INDIVIDUAL, PARTNERSHIP, ASSOCIATION OR CORPORATION RESIDENT VENDOR WHO HAS MAINTAINED ITS HEADQUARTERS OR PRINCIPAL PLACE OF BUSINESS CONTINUOUSLY IN WEST VIRGINIA FOR FOUR (4) YEARS IMMEDIATELY PRECEDING THE DATE OF THIS CERTIFICATION; OR

( ) BIDDER IS A CORPORATION NONRESIDENT VENDOR WHICH HAS AN AFFILIATE OR SUBSIDIARY WHICH EMPLOYS A MINIMUM OF ONE HUNDRED STATE RESIDENTS AND WHICH HAS MAINTAINED ITS HEAQUARTERS OR PRINCIPAL PLACE OF JUSINESS WITHIN WEST VIRGINIA CONTINUOUSLY FOR THE FOUR (4) YEARS IMMEDIATELY PRECEDING THE DATE OF THIS CERTIFICATION.

B. APPLICATION IS MADE FOR 2.5% PREFERENCE FOR THE REASON CHECKED:

( ) BIDDER IS A RESIDENT VENDOR WHO CERTIFIES THAT, DURING THE LIFE OF THE CONTRACT, ON AVERAGE AT LEAST 75% OF THE EMPLOYEES WORKING ON THE PROJECT BEING BID ARE RESIDENTS OF WEST VIRGINIA WHO HAVE RESIDED IN THE STATE CONTINUOUSLY FOR THE TWO YEARS IMMEDIATELY PRECEDING SUBMISSION OF THIS BID;
OR
( ) BIDDER IS A NONRESIDENT VENDOR EMPLOYING A MINIMUM OF ONE HUNDRED STATE RESIDENTS OR IS A NONRESIDENT VENDOR WITH AN AFFILIATE OR SUBSIDIARY

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE [signature] TELEPHONE 800-456-1157 EXT 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

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State of West Virginia
Department of Administration
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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
1450 Waukegan Road
McGraw Park, IL 60085

SHIP TO
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167-ELEVENTH AVENUE
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WHICH MAINTAINS ITS HEADQUARTERS OR PRINCIPAL PLACE OF BUSINESS WITHIN WEST VIRGINIA EMPLOYING A MINIMUM OF ONE HUNDRED STATE RESIDENTS WHO CERTIFIES THAT, DURING THE LIFE OF THE CONTRACT, ON AVERAGE AT LEAST 75% OF THE EMPLOYEES OR BIDDERS' AFFILIATE'S OR SUBSIDIARY'S EMPLOYEES ARE RESIDENTS OF WEST VIRGINIA WHO HAVE RESIDED IN THE STATE CONTINUOUSLY FOR THE TWO YEARS IMMEDIATELY PRECEDING SUBMISSION OF THIS BID.

BIDDER UNDERSTANDS IF THE SECRETARY OF TAX & REVENUE DETERMINES THAT A BIDDER RECEIVING PREFERENCE HAS FAILED TO CONTINUE TO MEET THE REQUIREMENTS FOR SUCH PREFERENCE, THE SECRETARY MAY ORDER THE DIRECTOR OF PURCHASING TO: (A) RESCIND THE CONTRACT OR PURCHASE ORDER ISSUED; OR (B) ASSESS A PENALTY AGAINST SUCH BIDDER IN AN AMOUNT NOT TO EXCEED 5% OF THE BID AMOUNT AND THAT SUCH PENALTY WILL BE PAID TO THE CONTRACTING AGENCY OR DEDUCTED FROM ANY UNPAID BALANCE ON THE CONTRACT OR PURCHASE ORDER.

BY SUBMISSION OF THIS CERTIFICATE, BIDDER AGREES TO DISCLOSE ANY REASONABLY REQUESTED INFORMATION TO THE PURCHASING DIVISION AND AUTHORIZES THE DEPARTMENT OF TAX AND REVENUE TO DISCLOSE TO THE DIRECTOR OF PURCHASING APPROPRIATE INFORMATION VERIFYING THAT BIDDER HAS PAID THE REQUIRED BUSINESS TAXES, PROVIDED THAT SUCH INFORMATION DOES NOT CONTAIN THE AMOUNTS OF TAXES PAID NOR ANY OTHER INFORMATION DEEMED BY THE TAX COMMISSIONER TO BE CONFIDENTIAL.

UNDER PENALTY OF LAW FOR FALSE SWEARING (WEST VIRGINIA CODE 61-5-3), BIDDER HEREBY CERTIFIES THAT THIS CERTIFICATE IS TRUE AND ACCURATE IN ALL RESPECTS; AND THAT IF A CONTRACT IS ISSUED TO BIDDER AND IF ANYTHING CONTAINED WITHIN THIS CERTIFICATE CHANGES DURING THE TERM OF THE CONTRACT, BIDDER WILL NOTIFY THE PURCHAS-

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE	TELEPHONE	DATE
Kenley Krum	800-456-1157 Ext 5139	9/4/06

TITLE	FEIN	ADDRESS CHANGES TO BE NOTED ABOVE
SP Representative	36-4095186	

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Request for Quotation

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ADDRESS CORRESPONDENCE TO ATTENTION OF:
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304-558-0067

VENDOR
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McGraw Park, IL 60085

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ING DIVISION IN WRITING IMMEDIATELY.

BIDDER: Cardinal Health

DATE: 9/4/06

SIGNED: Debby Ahrens

TITLE: S/P Representative

* CHECK ANY COMBINATION OF PREFERENCE CONSIDERATION(S) IN EITHER "A" OR "B", OR BOTH "A" AND "B" WHICH YOU ARE ENTITLED TO RECEIVE. YOU MAY REQUEST UP TO THE MAXIMUM 5% PREFERENCE FOR BOTH "A" AND "B".
(REV. 12/00)

NOTICE

AN ORIGINAL, SIGNED BID MUST BE SUBMITTED TO:

DEPARTMENT OF ADMINISTRATION
PURCHASING DIVISION
BUILDING 15
2019 WASHINGTON STREET, EAST
CHARLESTON, WV 25305-0130

SEALED BID

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Debby Ahrens TELEPHONE 800-456-1157 EXT 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

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LINE	QUANTITY	UOP	CAT. NO.	ITEM NUMBER	UNIT PRICE	AMOUNT

BUYER: RW-22
RFQ. NO.: LBS70424
BID OPENING DATE: 9/05/06
BID OPENING TIME: 1:30 P.M.

PLEASE PROVIDE A FAX NUMBER IN CASE IT IS NECESSARY TO CONTACT YOU REGARDING YOUR BID:

FAX # 513-844-1241

CONTACT PERSON: James Martin
Diagnostic Specialist
Cardinal Health

THE MODEL/BRAND/SPECIFICATIONS NAMED HEREIN ESTABLISH THE ACCEPTABLE LEVEL OF QUALITY ONLY AND ARE NOT INTENDED TO REFLECT A PREFERENCE OR FAVOR ANY PARTICULAR BRAND OR VENDOR. VENDORS WHO ARE BIDDING ALTERNATES SHOULD SO STATE AND INCLUDE PERTINENT LITERATURE AND SPECIFICATIONS. FAILURE TO PROVIDE INFORMATION FOR ANY ALTERNATES MAY BE GROUNDS FOR REJECTION OF THE BID. THE STATE RESERVES THE RIGHT TO WAIVE MINOR IRREGULARITIES IN BIDS OR SPECIFICATION IN ACCORDANCE WITH SECTION 148-1-4(F) OF THE WEST VIRGINIA LEGISLATIVE RULES AND REGULATIONS.

INQUIRIES
WRITTEN QUESTIONS SHALL BE ACCEPTED THROUGH CLOSE OF BUSINESS ON AUGUST 15, 2006. QUESTIONS

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wesley Brown TELEPHONE 800-456-1152 Ext 5139 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
LBS70424	11

ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
1450 Waukegan Road
McGraw Park, IL 60085

SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
08/01/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT NO.	ITEM NUMBER	UNIT PRICE	AMOUNT

MAY BE SENT VIA USPS, FAX, COURIER OR EMAIL. IN ORDER TO ASSURE NO VENDOR RECEIVES AN UNFAIR ADVANTAGE, NO SUBSTANTIVE QUESTIONS WILL BE ANSWERED ORALLY. IF POSSIBLE, E-MAIL QUESTIONS ARE PREFERRED. ADDRESS INQUIRIES TO:

ROBERTA WAGNER
DEPARTMENT OF ADMINISTRATION
PURCHASING DIVISION
2019 WASHINGTON STREET, EAST
CHARLESTON, WV 25311
FAX: 304-558-4115
E-MAIL: RWAGNER@WVADMIN.GOV

PLEASE NOTE THAT WE PREFER FOB DESTINATION, IF YOU WISH TO BID OTHER THAN FOB DESTINATION, YOU MUST PROVIDE THE MAXIMUM SHIPPING COST SO THAT IT CAN BE ADDED TO THE BID.

PREPAY TRANSPORTATION AND ADD TO INVOICE AS A SEPARATE ITEM. IF BY FREIGHT, ATTACH ORIGINAL PREPAID FREIGHT BILL.

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wesley Abrams TELEPHONE 800-456-1157 EXT 5139 DATE 9/4/06

TITLE S/R Representative FEIN 31-4095786

ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
LBS70424	12

ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR
Cardinal Health
Scientific Products Distribution
1450 Waukegan Road
McGraw Park, IL 60085

SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
08/01/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT NO.	ITEM NUMBER	UNIT PRICE	AMOUNT
	****** THIS IS THE END OF RFQ LBS70424 ****** TOTAL:					41,792.90

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wendy Allen TELEPHONE 800-456-1157 Ext 3739 DATE 9/4/06

TITLE S/P Representative FEIN 36-4095786

ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

LBS70424
SPECIFICATIONS

A. REAGENT SPECIFICATIONS

1. Reagents must be bar-coded.
2. Must have ability to support up to 31 controls and calibrators.
3. Must be able to support multi-size reagent bottle volumes (milliliters) 3,8,15,30,60,125.
4. Must have lot and expiration management for reagents.
5. Must have disposable graphite tips, 300-1000 microliters.
6. Must have at least an on-board capacity of 480 tips (5 boxes of 96 tips).
7. Must be able to track tip usage on instrument and use partially filled tip racks.
8. Must have tip waste capacity of >1000 tips.
9. Must have mechanical tip detection.
10. Must have liquid level and clot detection.
11. Positive sample identification must be on plates and reagents.
12. The test kit must have a minimum shelf life of 90 days or more beyond date of receipt.

Assay Specifications for HIV-1 and HIV-2 plus O Group

1. Must detect antibodies to HIV-1 and 2 plus Group O in serum, plasma and cadveric samples.
2. Must meet the following criteria for HIV ½ recombinant DNA/synthetic peptide assay.
   a. Must have a 96-well test plate.
   b. HIV ½ peptide kit must have the following:
      1). Synthetic Peptide Immunoassay for the detection of the antibody to
      2). HIV-1 and HIV-2. The microtiter wells are coated with a mixture of peptides; env and pol sequences for HIV-1 and HIV-2.
      3). Sample dilution 1/10.
      4). Must be FDA licensed recombinant peptide EIA for HIV-1 and HIV-2 plus Group O.
3. The principle must be direct antibody, sandwich Elisa in a solid phase microwell.
4. Sample size must not be greater than 75ul.
5. Turnaround time must not be greater than 3 hours for the HIV assay.
6. All steps in the method must be automated, including data reduction on one primary microplate instrument.
7. Incubation times (~~on the Evolis instrument~~) must not exceed (in minutes) 60-30-30. ~~instrument~~
8. Chromogen should not be lot specific for kit.
9. Stop solution must be ready to use.

LBS70424
SPECIFICATIONS

Test Type Description	Brand Product # or Equal	Estimated Annual Usage	Unit Cost	Total Cost
Hepatitis A	Diasorin Anti-HAV IgM #P001925	100	6.01	601.00
Hepatitis B	Genetic Systems HBsAg Confirmatory #32594	10	5.99	59.90
Hepatitis B	Genetic Systems HBsAg 3.0 EIA #32591	2000	4.30	8595.00
Hepatitis B	Diasorin Anti-HBCore #P001927	2000	4.98	9956.00
Hepatitis B	Diasorin Anti-HBc IgM #P001928	100	5.97	597.00
Hepatitis B	DiaSorin Anti-HBS	1000	4.89	4,889.00
Hepatitis C	Ortho HCV EIA v3.0 #930740	2000	8.55	17,095.00
HIV	BioRad HIV 1/2/O #32588	7000	no bid	

B. INSTRUMENT SPECIFICATIONS

General Instrument Specifications

1. Must have primary sample capacity of 180 samples.
2. Must have 20 tubes per sample linear rack.
3. Must have Positive Identification for samples, microplates and reagents.
4. Must be able to sample from tubes up to 16mm diameter
5. Must be able to sample from tubes up to 100mm height.
6. Dead volume can not be greater than 200 microliters.
7. All sample positions must be bar-coded on the sample tube and sample rack.
8. All reagent and quality control racks must be bar-coded.
9. Must have dilution capacity via tubes and microplate.
10. Sample diltuion must be 1:10,000 or less.
11. Must have the capability to load continuously throughout the sample processing.
12. Must include computer system and software.
13. Must be able to shake assays for variable times.
14. Must be able to process blood virus, infectious disease and autoimmune in a single run.
15. Must be able to incubate assays at Room Temperature (R.T.) and at 37 degrees C.

Washer Specifications

1. Must have an 8 channel manifold.
2. Must be able to use flat, U and V shaped plate bottom shapes.
3. Must have a plate and strip wash mode.
4. Must have variable wash cycles of 1 to 9.
5. Must have plate soak time of 0-999 secs.
6. Must have wash buffers with level sensors of 2 x 2L and 2 x 1L.
7. Must have waste capacity with level sensors of 1 x 10L.

Reader Specifications

1. Must have 8 channel read head.
2. Read time for full plate must not be greater than 15 seconds.
3. Must have a halogen light source.
4. Must have a reading range of up to 3.5 Optical Density (O.D.)
5. Must be equipped with at least 8 filter wheels to include 405,450, 492, 550, 620, 690 nm.
6. Must have an over-range filter.
7. Must have linearity (0-3.0 O.D.) to 1%; precision (0-2.0 O.D.) to 2.5%.

C. COMPUTER INTERFACE SPECIFICATIONS

1. Must have ability to connect multiple ~~Evolis~~ Instruments (up to 8) to a LAN (Local Area Network) and use one computer interface to interface to the facilities LIMS (Laboratory Information Management System) provider.
2. Interface cost must be included in cost per test.
3. Must have bi-directional interface with ASTM or ASCH file format.
4. User interface must be Windows 2000 operating system.
5. Must be able to track reactive results and perform duplicate assays prior to confirmation.
6. Must create a Primary Sample Validation Screen and Worklist for reactive samples.
7. Must be able to process and send repeat Worklist to ~~Evolis~~ Instrument for analysis.
8. Must be able to check assay results to see if they are final (repeat reactive) or require repeating (initially reactive).
9. Once all results (both initial and repeat reactive) have been validated by the system, the final results must have the capability of being exported to the LIMS.
10. Vendor must be willing to assist in transition process to the LIMS.

D. TRAINING / INSTALLATION REQUIREMENTS

1. Vendor must provide a company representative for installation and training. Subcontracting of these services shall not be acceptable to the State of West Virginia. Any vendor responding to this contract that proposes to utilize a subcontractor shall not be considered during the award process.
2. Installation and training for equipment must be completed within six (6) weeks of delivery date and must include one (1) key operator training at vendor's training site at vendor's expense and training of other staff members at OLS facility also at vendor's expense.

LBS70424
SPECIFICATIONS

E. EQUIPMENT OWNERSHIP / MAINTENANCE / TECHNICAL ASSISTANCE REQUIREMENTS

1. Vendor will retain ownership of all instrumentation
2. All instrumentation provided by the selected vendor must be maintained at vendor's expense during the term of this contract. One (1) annual preventive maintenance visit at the laboratory site must be provided at no additional charge.
3. Vendor must provide a company representative for technical service, repairs, maintenance, etc. Any vendor responding to this contract that proposes to utilize a subcontractor shall not be considered during the award process.
4. Technical assistance must be available by telephone during normal business hours, 8:00 a.m. to 5:00 p.m. EST, Monday through Friday. If technical assistance does not resolve problems, replacement parts or loaner modules must be provided or on-site representative presence must be made available within 24 hours, except on weekends.

DELIVERY / SHIPPING REQUIREMENTS

1. To be F.O.B. Destination, unless vendor states otherwise in submitted quotation.
2. Reagents must be shipped no more than 3 days after receiving order.

LIFE OF CONTRACT:

This contract is to become effective October 16, 2006 and extends for a period of one (1) year, or until such "reasonable time" thereafter as is necessary to obtain a new contract. At the end of one (1) year, an option is reserved to renew the agreement in accordance with the terms and conditions of the original contract and shall be limited to two (2) one (1) year periods.

ORDERING PROCEDURE:

Spending unit shall issue a written state contract order (Form Number WV-39) to the vendor for commodities covered by this contract, except when purchases are of a dollar amount allowable to be made with the WV State Credit Card (P-Card).

RFQ No. LBS70424

AFFIDAVIT

West Virginia Code §5A-3-10a states:
No contract or renewal of any contract may be awarded by the state or any of its political subdivisions to any vendor or prospective vendor when the vendor or prospective vendor or a related party to the vendor or prospective vendor is a debtor and the debt owned is an amount greater than one thousand dollars in the aggregate.

DEFINITIONS:
"Debt" means any assessment, premium, penalty, fine, tax or other amount of money owed to the state or any of its political subdivisions because of a judgment, fine, permit violation, license assessment, defaulted workers' compensation premium, penalty or other assessment presently delinquent or due and required to be paid to the state or any of its political subdivisions, including any interest or additional penalties accrued thereon.

"Debtor" means any individual, corporation, partnership, association, limited liability company or any other form or business association owing a debt to the state or any of its political subdivisions.

"Political subdivision" means any county commission; municipality; county board of education; any instrumentality established by a county or municipality; any separate corporation or instrumentality established by one or more counties or municipalities, as permitted by law; or any public body charged by law with the performance of a government function or whose jurisdiction is coextensive with one or more counties or municipalities.

"Related party" means a party, whether an individual, corporation, partnership, association, limited liability company or any other form or business association or other entity whatsoever, related to any vendor by blood, marriage, ownership or contract through which the party has a relationship of ownership or other interest with the vendor so that the party will actually or by effect receive or control a portion of the benefit, profit or other consideration from performance of a vendor contract with the party receiving an amount that meets or exceed five percent of the total contract amount.

EXCEPTION:
The prohibition of this section does not apply where a vendor has contested any tax administered pursuant to chapter eleven of this code, workers' compensation premium, permit fee or environmental fee or assessment and the matter has not become final or where the vendor has entered into a payment plan or agreement and the vendor is not in default of any of the provisions of such plan or agreement.

LICENSING:
The vendor must be licensed in accordance with any and all state requirements to do business with the state of West Virginia.

CONFIDENTIALITY:
The vendor agrees that he or she will not disclose to anyone, directly or indirectly, any such personally identifiable information or other confidential information gained from the agency, unless the individual who is the subject of the information consents to the disclosure in writing or the disclosure is made pursuant to the agency's policies, procedures and rules. Vendors should visit **www.state.wv.us/admin/purchase/privacy** for the Notice of Agency Confidentiality Policies.

Under penalty of law for false swearing (West Virginia Code, §61-5-3), it is hereby certified that the vendor acknowledges the information in this said affidavit and are in compliance with the requirements as stated.

Vendor's Name: Cardinal Health

Authorized Signature: Kimberly Abrams Date: 9/4/06

No Debt Affidavit
Revised 02/08/06

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
LBS70424	1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR:
RFQ COPY
TYPE NAME/ADDRESS HERE

SHIP TO:
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
08/18/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT NO.	ITEM NUMBER	UNIT PRICE	AMOUNT

**********ADDENDUM NO. 2**********

1. TO RESPOND TO QUESTION(S) ASKED.

QUESTION: VENDOR WOULD LIKE TO KNOW IF THE HIV TEST THEY CAN PROVIDE WHICH IS 100% SPECIFIC AND 99% SENSITIVE AND DETECTS HIV 1 AND 2 BUT ONLY CLAIMS HIV 1 IS ACCEPTABLE.

ANSWER: SPECIFICATIONS REQUIRE THE DETECTION OF HIV 1 AND 2. ALL BIDS RECEIVED MUST BE SUBSTANCIATED TO MEET OR EXCEED THESE SPECIFICATIONS. ALL VENDORS MUST PROVIDE SUPPORTING DOCUMENTATION THAT QUALIFIES THEY ARE BIDDING AN APPROVED TEST FOR DETECTION OF BOTH HIV 1 AND 2.

2. ADDENDUM ACKNOWLEDGEMENT IS ATTACHED. THIS DOCUMENT SHOULD BE SIGNED AND RETURNED WITH YOUR BID. FAILURE TO SIGN AND RETURN MAY RESULT IN DISQUALIFICATION OF YOUR BID.

PLEASE NOTE THE FOLLOWING ATTACHMENT:
1) ADDENDUM ACKNOWLEDGEMENT

**********END OF ADDENDUM NO. 2**********

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Debbie Wagner TELEPHONE 800-456-1157 Ext 5139 DATE 9/5/06

TITLE SP Representative FEIN 36-4095186 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

WV-36a STATE OF WEST VIRGINIA **PURCHASING CONTINUATION SHEET**	Buyer:	Page	Req. or P. O. No.:
Vendor:	Spending Unit:		

Requisition No.: LBS70424

ADDENDUM ACKNOWLEDGEMENT

I hereby acknowledge receipt of the following checked addendum(s) and have made the necessary revisions to my proposal, plans and/or specifications, etc.

Addendum No.'s:

No. 1 DD

No. 2 DD

No. 3 _____

No. 4 _____

No. 5 _____

I understand that failure to confirm the receipt of the addendum(s) is cause for rejection of bids.

Signature

Cardinal Health
Company

9/5/06
Date

Exhibit 10
Rev. 11/96

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Request for Quotation

RFQ NUMBER	PAGE
LBS70424	1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
ROBERTA WAGNER
304-558-0067

VENDOR
RFQ COPY
TYPE NAME/ADDRESS HERE

SHIP TO
HEALTH AND HUMAN RESOURCES
BPH - LABORATORY SERVICES

167-ELEVENTH AVENUE
SOUTH CHARLESTON, WV
25303 304-558-3530

DATE PRINTED	TERMS OF SALE	SHIP VIA	F.O.B.	FREIGHT TERMS
08/11/2006				

BID OPENING DATE: 09/05/2006 BID OPENING TIME 01:30PM

LINE	QUANTITY	UOP	CAT NO.	ITEM NUMBER	UNIT PRICE	AMOUNT

PLEASE MAKE THE FOLLOWING CHANGES TO THE REQUISITION:
PAGE 1, 1ST PARAGRAPH, LINE 6: TO READ: "WITH" INSTEAD OF: "WIT"

PAGE 1, ITEM 1- TO READ: "DIASORIN" INSTEAD OF "DIASOIN"

PAGE 13-A. ASSAY SPECIFICATION,
ITEM #7-DELETE "(ON THE EVOLIS INSTRUMENT)"

PAGE 15 - C. COMPUTER INTERFACE SPECIFICATIONS
ITEM #1-DELETE THE WORD "EVOLIS" AND INSERT "INSTRUMENTS"
ITEM #7- DELETE THE WORD "EVOLIS" AND INSERT "THE INSTRUMENT"

ADDENDUM ACKNOWLEDGEMENT IS ATTACHED. THIS DOCUMENT SHOULD BE SIGNED AND RETURNED WITH YOUR BID. FAILURE TO SIGN AND RETURN MAY RESULT IN DISQUALIFICATION OF YOUR BID.

PLEASE NOTE THE FOLLOWING ATTACHMENT:
1) ADDENDUM ACKNOWLEDGEMENT

**********END OF ADDENDUM NO. 1**********

SEE REVERSE SIDE FOR TERMS AND CONDITIONS

SIGNATURE Wesley Wrenn TELEPHONE 800-456-1157 Ext 5139 DATE 9/5/06

TITLE S/P - Cardinal Health FEIN 36-4095186

ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO RFQ, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

WV-36a **STATE OF WEST VIRGINIA** **PURCHASING CONTINUATION SHEET**	Buyer:	Page	Req. or P. O. No.:
Vendor:	Spending Unit:		

Requisition No.: LBS70424

ADDENDUM ACKNOWLEDGEMENT

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Addendum No.'s:

No. 1 DD

No. 2 _____

No. 3 _____

No. 4 _____

No. 5 _____

I understand that failure to confirm the receipt of the addendum(s) is cause for rejection of bids.

Signature

Cardinal Health
Company

9/5/06
Date

Exhibit 10
Rev. 11/96

CardinalHealth

DiaSorin

August 30, 2006

Roberta Wagner
State of West Virginia Purchasing Division
2019 Washington Street East
Charleston, WV 25311

Dear Roberta:

The enclosed information is in response to RFQ number LBS70424.

In response, Cardinal Health and DiaSorin is proposing the ETI-MAX 3000 which is in compliance with the specifications indicated in the RFQ.
The DiaSorin ETI-MAX 3000 is validated to perform all of the tests request in the RFQ.
The HBsAG and the HBsAg confirmatory assays will be substituted with the equivalent DiaSorin assays. The DiaSorin ETI-MAX is validated by the manufacturer for the performance of all DiaSorin assays, the Ortho HCV assay and the Bio-Rad HIV 1/2/0 assay.
The Bio-Rad HIV assay will have to be purchased from Bio-Rad directly.

Thank you for the opportunity to present this proposal in response to your RFQ.

Regards,

James Martin
Diagnostic Specialist
Cardinal Health
Scientific Products Distribution
800 456 1157 x 5550
CP 513 518 1161

ETI-MAX™ 3000
MAXIMIZE YOUR ANALYSIS

ETI-MAX™ 3000 FEATURES

- High Throughput – 4 plates up to 7
- Up to 180 sample tubes onboard at the same time
- Continuously load samples and reagents
- Bar-coded samples and reagents reduce errors
- Clot & liquid level detection for samples & reagents
- Easy Maintenance – Automatic daily and weekly
- Disposable tips – no carryover

SOFTWARE

- Bi-Directional interface capability
- Multi-Task (multiple SW functions during the run)
- Windows software based functionality

Cardinal meets the challenge of making your laboratory microplate testing safe, simple and economical with the automated ETI-MAX 3000 system.

ETI-MAX 3000 maximizes your laboratory performance by offering high throughput, safety, and reliable results

DiaSorin

CardinalHealth

ETI-MAX 3000™ SOLUTION

The ETI-MAX 3000™ is a fully automated microplate analyzer designed to simplify and optimize your routine laboratory. It can simultaneously load up to four microplates with separate work lists and can process up to seven microplates in continuous loading. DiaSorin meets the challenge of making your microplate routine, simple, and safe. Bar-code reading for primary tubes and DiaSorin reagents handled by DiaSorin's next generation software ensures reliability and reduces operator's errors. DiaSorin's ETI-MAX 3000™ offers a true walk-away automated system that controls and records all the operations performed by the instrument, guaranteeing reliable results with DiaSorin's renowned tradition.

Technology

- Fully automated microplate analyzer
- Four microplates simultaneously; up to seven microplates with its continuous loading capability
- Walk-away system
- State-of-the-art software operates under Windows® program
- Complete sample processing (sample predilutions, sample dispensing, reagent dispensing, incubations, wash processes and plate transports

Versatile and productive

- On-line dilution capabilities, including ability to perform serial dilutions
- Disposable tips, designed for clot detection and level sensing
- Handles up to 180 samples onboard the system
- Random access and batch mode: multiple analytes can be run on same plate
- Up to four microplates run simultaneously; up to seven microplates with the continuous load function
- Additional plates (assays/patients) may be added even after the run has been started

Operating efficiency

- Instrument automatically calculates tips, tubes and reagents necessary for assay
- System scheduler calculates the actual run of the system and real-time scheduler allows user to track progress of the run
- Positive bar-code recognition for both samples and reagents
- Reagent racks designed for the DiaSorin reagents
- Continuous loading capability
- High-dispensing precision, clot detection and no carry over with disposable conductive tips

Software

- User-friendly software is Windows® based software
- Bidirectional LIS interface capability
- If not using an LIS, reports are flexible by patient, date, assay type, etc.
- Flexibility in protocol programming and creation of microplate maps
- Unlimited number of protocols
- Schedule for optimizing loading times
- Data reduction: manages different types of calculations
- Levey-Jennings for checking kit and quality control
- Multitasking: multiple software functions available during the run

Safety characteristics

- Selftest capabilities to verify all system functions upon initialization
- On-line in-process control: all instrument operations and/or errors are recorded and can be printed out during tests
- Bar codes for primary tubes, reagents and microplates for identification
- Automatic closing of stations: software opens different stations according to current step in procedure
- Automatic checking of required reagent volumes and verification of strip presence

Quality control and calibration

- Quality control and calibrators are run with each plate
- Kits contain their own controls (usually positive/negative) and some customers will run external controls

CardinalHealth

DiaSorin

CardinalHealth

ETI-MAX 3000™ Assays

We can provide more than fifty ELISA microtiter plate assays, including an extensive menu of Hepatitis, Infectious Disease and Autoimmune tests.

Hepatitis			
IgM Anti-HAV	**B1032-270**/96 Wells	Anti-HBs	**B1032-276**/192 Wells
Anti-HAV	**B1032-271**/96 Wells	Anti-HBs Quant Std. Set	**B1032-278**/10 runs
Anti-HBc	**B1032-272**/192 Wells	HBsAg	**B1032-349**/192 Wells
IgM Anti-HBc	**B1032-273**/96 Wells	HBsAg Confirmatory	**B1032-350**/44 Tests
Anti-HB(e) Antibody	**B1032-274**/96 Wells	Ortho® Anti-HCV 3.0	**B1032-281**/480 Wells
HB(e) Antigen	**B1032-275**/96 Wells		

Infectious Disease			
EBNA-IgG	**B1032-265**/96 Wells	Lyme IgG & IgM Combo	**B1032-371**/96 Wells
VCA-IgG	**B1032-264**/96 Wells	HSV I/II IgG	**B1032-46**/96 wells
VCA-IgM Reverse Capture	**B1032-264**/96 Wells	Trep-Chek™ Syphilis IgG	**B1032-40**/96 wells
EA (D) IgG	**B1032-78**/96 Wells	Trep-Chek™ Syphilis IgG	**B1032-41**/960 wells
Measles IgG	**B1032-360**/96 Wells	CMV IgG	**B1032-44**/96 wells
Varicella Zoster IgG	**B1032-363**/96 Wells	CMV IgM Capture	**B103-45**/96 wells
Mumps IgG	**B1032-370**/96 Wells	Toxoplasma IgG	**B1032-42**/96 wells
Rubella IgG	**B1032-267**/192 wells	Toxoplasma IgM Capture	**B1032-43**/96 wells
H. Pylori IgG	**B1032-372**/96 Wells	Vironostika® HTLV I/II	**B1032-380**/192 wells

Autoimmune Disease			
ANA Screen Kit	**B1032-69**/96 Wells	Anti-Mitochondrial	**B1032-355**/96Wells
ENA 6 Screen Kit	**B1032-70**/96 Wells	Anti-Cardiolipin Total Kit	**B1032-354**/96Wells
Anti-dsDNA Kit	**B1032-71**/96 Wells	Anti-B2 Glycoprotein 1	**B1032-47**/96Wells
Anti-Sm Kit	**B1032-72**/96 Wells	Anti-Thyroglobulin	**B1032-366**/96Wells
Anti-Sm/RNP Kit	**B1032-73**/96 Wells	Anti-Thyroid Peroxidase(TPO)	**B1032-367**/96Wells
Anti-SS-B Kit	**B1032-74**/96 Wells	Anti-Cardiolipin IgA	**B1032-780**/96 Wells
Anti-SS-A Kit	**B1032-75**/96 Wells	Anti-Cardiolipin IgG	**B1032-79**/96 Wells
Anti-Jo-1 Kit	**B1032-76**/96 Wells	Anti-Cardiolipin IgM	**B1032-80**/96 Wells
Anti-Scl-70 Kit	**B1032-77**/96 Wells	Anti-CCP	**B1032-351**/96 Wells

DiaSorin

CardinalHealth

Confidential

DiaSorin Diagnostic Support

DiaSorin standard instrument service includes with instrument:

- First year covered under manufacturer's warranty
- Onsite assistance available Monday through Friday 8:00 a.m. to 5:00 p.m.
- Hot line available 7 a.m. to 7 p.m. CST, 800.328.1482
- Emergency replacement parts available:
  basic parts provided
  additional parts shipped priority on an as as needed basis
- Preventive maintenance visits every six months (average downtime approximately six h

CardinalHealth

Implementation and training

Upon acceptance of your purchase order, you may expect the following to occur, which will ensure expedient implementation of your systems.

Support	Description
Instrumentation installation	DiaSorin will install and set up instrumentation in the laboratory. Installation will be performed before training.
Implementation checklist	Checklist provides actions and dates for implementing the system into the laboratory.
Training	Training for two key operators can either be held on site or DiaSorin also allows the option of training at their facility in Stillwater, MN.
Training checklist	The checklist provides the following: Learning objectives, competency assessment, training schedule; procedure manuals and crossover assistance
Training cost information	When training option in Stillwater, MN is chosen; included is the airline tickets, hotel accomodations and a daily meal per diem for two key operators. This cost is included in the purchase/service agreement. Additional training is available at an additional cost.

HBsAg
Confirmatory Test

Neutralization Assay for Confirmation of
Hepatitis B Surface Antigen (HBsAg)
in Repeatedly Reactive Human Serum and Plasma Samples
as Determined by ETI-MAK-2 PLUS

Instruction Manual

Catalog No.: N0143

Manufactured By:

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Saluggia, Italy

Distributed By:

DiaSorin Inc.
Stillwater, MN, USA

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1. INTENDED USE

The DiaSorin HBsAg Confirmatory Test is an in vitro neutralization assay for qualitative confirmation of the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma samples (EDTA, heparin, and citrate) found to be repeatedly reactive for HBsAg by ETI-MAK-2 PLUS. This assay has not been FDA-approved for the screening of blood or plasma donors.

Assay performance characteristics have not been established when the DiaSorin HBsAg Confirmatory Test is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Assay performance characteristics have not been established for newborn testing.

2. SUMMARY AND EXPLANATION OF THE TEST

In spite of the high specificity achieved with test methods, the possibility exists that falsely reactive results may be encountered due to the presence of non-specific interfering substances, artifacts in the reagents, or the type of methodology used. To reduce this possibility, neutralization tests were developed to ensure that HBsAg-reactive results are caused by the presence of the surface antigen and not by non-specific interference.

The DiaSorin HBsAg Confirmatory Test is comprised of Antibody to HBsAg (Human) and Specimen Diluent (Human) and is used in conjunction with ETI-MAK-2 PLUS to test all repeatedly reactive samples. All samples that are repeatedly reactive for HBsAg by ETI-MAK-2 PLUS and that are neutralized by Antibody to HBsAg are considered confirmed as positive for HBsAg. Samples that are repeatedly reactive for HBsAg and that are not neutralized by Antibody to HBsAg are considered as negative for HBsAg.

3. PRINCIPLE OF THE PROCEDURE

The HBsAg Confirmatory Test is based on the principle of binding inhibition or neutralization of binding activity.

A neutralizing reagent containing human antibodies to HBsAg is added to one aliquot of each specimen found repeatedly reactive (neutralized aliquot). As a control procedure, anti-HBs-negative human serum (Sample Diluent) is added to the other aliquot (non-neutralized or test aliquot). When the neutralizing reagent has been added to a sample containing HBsAg, the antibodies in the neutralizing reagent bind to the HBsAg, forming antigen-antibody complexes. When the neutralizing reagent has been added to a sample containing a non-specific reactive substance, the antibodies in the neutralizing reagent will not bind to this non-specific substance. The aliquots are then tested using the Dia Sorin ETI-MAK-2 PLUS Assay. If the sample contains HBsAg, the antigen-antibody complexes formed with the neutralizing reagent inhibit the HBsAg from reacting in the ETI-MAK-2 PLUS assay. If the sample contains non-specific substances, no complexes are formed and the substance is then available for reacting in the HBsAg assay.

An absorbance reading of a neutralized aliquot that is significantly lower than that of the corresponding non-neutralized aliquot demonstrates HBsAg neutralization and the presence of HBsAg in the sample is confirmed.

Refer to ETI-MAK-2 PLUS package insert for a description of the principle of the procedure.

4. REAGENTS PROVIDED

Catalog Number	Product Description	Quantity/ Volume
N0143	HBsAg Confirmatory Test*	
	Specimen diluent Human serum/plasma non-reactive for HBsAg and anti-HBs (ready to use). Preservative: 0.2% ProClin 300.	1 vial, 15 mL
	Anti-HBs (Polyclonal human antibody to Hepatitis B Surface Antigen) Human serum/plasma containing ≥ 20,000 mIU/mL anti-HBs antibody referenced to WHO Anti-Hepatitis B Immunoglobulin 1st International Reference Preparation (1977), an inert red dye (ready to use). Preservative: 0.2% ProClin 300.	1 vial, 0.7 mL

* *The reagents are sufficient for a maximum of 44 specimens plus controls.*

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- The human blood source material used to prepare this product derives from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 (AIDS) when tested by licensed screening tests, and found to be non-reactive for syphilis when tested by a serological test. Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, May 1999, and NCCLS Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue (1-3).
- All specimens, reagents, and controls should be handled as if capable of transmitting disease. Follow standard precautions for handling infectious agents during all procedures:
  - Do not pipette by mouth.
  - Do not eat, drink, smoke, or apply cosmetics in areas where specimens are handled.
  - Wear protective clothing such as lab coats, protective glasses, and disposable gloves when handling specimens and assay reagents. Wash hands thoroughly afterwards.
  - Perform all work with infectious materials in a designated area.
- Dispose of all specimens and used assay materials as if capable of transmitting disease:
  - Decontaminate liquid wastes, including those containing neutralized acid, either:
    (a) by autoclaving for 60 minutes at 121°C; or
    (b) by treating with a 1:10 or 1:100 dilution of household bleach (sodium hypochlorite concentration approximately 5%). The wastes should remain in contact with the sodium hypochlorite solution for 30 minutes for effective decontamination, after which they can be disposed of in the sink (1, 3). Do not autoclave solutions containing sodium hypochlorite.
  - Autoclave non-ignitable solids for 60 minutes at 121°C.
  - Incinerate disposable ignitable materials.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Use only dispensing equipment that has been calibrated to deliver accurate volumes, per the laboratory's standard procedures.
- For the ETI-MAK-2 PLUS components, observe all precautions as stated in section on Warnings and Precautions of that assay's package insert.

- **Warning** - *Reagents containing ProClin 300 may cause allergic reactions. Avoid prolonged contact with skin. Wash thoroughly after handling.*

6. REAGENT PREPARATION

- Bring the reagents to room temperature (20-25°C).
- For ETI-MAK-2 PLUS components, follow all preparation and storage instructions as stated in that assay's package insert.

7. REAGENT STORAGE AND HANDLING INSTRUCTIONS

- Store the reagents in the refrigerator at 2-8°C. Allow them to reach room temperature (20-25°C) before use. Return the test components to the refrigerator after use.
- Do not expose the test components to intense light, direct sunlight, or temperatures above 25°C. Do not freeze the kit.
- When test components are stored as directed, they will remain stable until expiration dates printed on their labels.
- Store ETI-MAK-2 PLUS components in the refrigerator at 2-8°C away from intense light. Allow them to reach room temperature (20-25°C) before use. Return the test components to the refrigerator after use.

8. REAGENT INSTABILITY OR DETERIORATION

- Any reagent that contains visible particulate matter should be discarded.

9. SPECIMEN COLLECTION AND PREPARATION

- All specimens found repeatedly reactive by ETI-MAK-2 PLUS must be confirmed by the DiaSorin HBsAg Confirmatory Test.
- This assay is not designed to test body fluids other than human serum or plasma. This assay is not designed for testing human cadaver fluids.
- Specimens containing precipitate may give inconsistent test results. Do not test specimens containing particulate material, or grossly hemolyzed or lipemic specimens.
- The testing of heat-inactivated samples is not recommended.
- Samples that are to be used fresh may be stored for up to two hours at 2-8°C in the presence of clots. Serum separated from the clot may be stored at 2-8°C up to 48 hours, but then must be frozen and stored deep-frozen (at –20°C or below) in sterile containers until use (4). If sample is stored frozen, mix thawed sample well before testing (vortex). It has been shown that up to three freeze-thaw cycles do not interfere with the assay.
- For shipping, specimens should be frozen at –20°C or below and shipped with dry ice. Temperature level during entire shipment should be no greater (warmer) than –20°C. Pack specimens in compliance with government regulations covering the transportation of etiologic agents (5).
- The HBsAg Confirmatory Test may be performed on human serum or plasma. EDTA, citrate or heparin anticoagulants have been tested and may be used with this assay. Follow manufacturer's instructions carefully when using plasma collection containers with anticoagulants.
- Repeatedly reactive specimens are assayed in duplicate (neutralized and non-neutralized aliquots). The minimum volume required for confirmation testing is 300 µL for specimens with absorbance within the reading range and 10 µL for specimens with absorbance above the reading range.

10. CONFIRMATION ASSAY PROCEDURE

Materials Provided

DiaSorin HBsAg Confirmatory Test
Specimen Diluent
Anti-HBs (Antibody to HBsAg).

Materials Required but not Provided

ETI-MAK-2 PLUS (Catalog no. P001932).

Microwell plate washer - The following instrument specifications are recommended for the performance of the HBsAg Confirmatory Test.
Volume dispensed: 350-370 µL
Number of wash cycles: 5
Soak time: 30 seconds
Aspirate the last aliquot of dispensed liquid: yes.

Note *- The volume of each microwell is approximately 400 µL. Make sure the volume of working wash buffer dispensed into each well does not cause the wells to overflow. If the wells overflow, set the washer to dispense less working wash buffer.*

Microwell plate reader - The following instrument specifications are recommended for the performance of the HBsAg Confirmatory Test.
Wavelength: dual wavelength, 450 nm and 600-650 nm
Bandwidth: ≤ 10 nm
Absorbance range: 0 absorbance units to reading range of reader (at least 2.5 absorbance units)
Repeatability: better than or equal to 0.005 absorbance units, or 1%, whichever is greater
Linearity or accuracy: better than or equal to 0.010 absorbance units, or 2%, whichever is greater
Drift: less than 0.005 absorbance units per hour.

Incubator, 37°C ± 1°C.

Note *- Gravity convection incubators are recommended. Forced-air incubators may cause edge effects. Do not use water baths as incubators.*

Micropipettes with disposable clean tips (15 µL, 50 µL, 100 µL and 150 µL).

Note *- Suggested specifications for micropipetters (based on gravimetric testing) are:*
≤ 50 µL: accuracy ± 3%, precision 2%
≥ 100 µL: accuracy ± 2%, precision 1%.

Miscellaneous clean glass or plastic containers
Hazardous waste disposal materials
Disposable gloves
Distilled or deionized water
Pipetter-diluter (optional)
Multichannel pipetter (optional)
Pipette tips for multichannel pipetter (if multichannel pipetter is used)
Disposable reagent reservoirs (if multichannel pipetter is used)
Printer compatible with microwell reader.

Neutralization Procedure

- Negative and positive controls for the ETI-MAK-2 kit:
  Mix 150 µL control and 15 µL Anti-HBs in one tube (neutralized aliquot = N) as well as 150 µL control and 15 µL Specimen Diluent in another tube (non-neutralized aliquot = T).
- Repeatedly reactive samples with absorbance within the reading range:
  Mix 150 µL specimen and 15 µL Anti-HBs in one tube (neutralized aliquot) as well as 150 µL specimen and 15 µL Specimen Diluent in another tube (non-neutralized aliquot).
- Repeatedly reactive samples with absorbance above the reading range:
  Dilute specimens 1:101 with Specimen Diluent (e.g., 5 µL specimen + 500 µL Specimen Diluent). The predilution may be stored at 2-8°C for 24 hours.
  Mix 150 µL diluted specimen and 15 µL Anti-HBs in one tube (neutralized aliquot) as well as 150 µL diluted specimen and 15 µL Specimen Diluent in another tube (non-neutralized aliquot).
- Incubate the specimens for 1-2 hours at room temperature (20-25°C). Treat specimens and controls in parallel.

Assay Procedure

The DiaSorin HBsAg Confirmatory Test is performed on all samples that test repeatedly reactive by the ETI-MAK-2 PLUS. Follow the assay procedure described in the ETI-MAK-2 PLUS package insert for testing the confirmatory aliquots. Calibrators, controls and samples are to be placed in wells as described in Table 1.

Table 1 - Configuration of Calibrators, Controls and Samples in Microwell Plate.

Well(s)	Calibrator, Control or Sample
A1	Blank well (containing only chromogen/substrate and stop solution).
B1, C1, D1	100 µL ETI-MAK-2 PLUS calibrator (untreated aliquot).
E1	100 µL ETI-MAK-2 PLUS negative control (untreated aliquot).
F1	100 µL ETI-MAK-2 PLUS positive control (untreated aliquot).
G1	100 µL ETI-MAK-2 PLUS negative control (non-neutralized aliquot = T).
H1	100 µL ETI-MAK-2 PLUS negative control (neutralized aliquot = N).
A2	100 µL ETI-MAK-2 PLUS positive control (non-neutralized aliquot = T).
B2	100 µL ETI-MAK-2 PLUS positive control (neutralized aliquot = N).
C2	100 µL HBsAg-reactive Sample 1 (non-neutralized aliquot = T).
D2	100 µL HBsAg-reactive Sample 1 (neutralized aliquot = N).
E2	100 µL HBsAg-reactive Sample 2 (non-neutralized aliquot = T).
F2	100 µL HBsAg-reactive Sample 2 (neutralized aliquot = N).
G2	100 µL HBsAg-reactive Sample 3 (non-neutralized aliquot = T).
H2	100 µL HBsAg-reactive Sample 3 (neutralized aliquot = N).
etc.	

Perform assay quality control procedures. Before evaluating results, perform quality control procedures (see Section 11, Quality Control).

11. QUALITY CONTROL

Refer to ETI-MAK-2 PLUS package insert for procedure to validate quality control (6, 7).

1. Evaluate the absorbance value of the substrate blank.

Blank the instrument on the well containing only chromogen/substrate and stop solution (see Step 14 in Section 10, Assay Procedure). The absorbance value for the blank well must be between 0.000 and 0.150 for the assay to be valid. If the absorbance value of the substrate blank is less than 0.000 or greater than 0.150, the run must be repeated.
***Note** - Subtract the substrate blank absorbance value from each absorbance value before performing the following evaluations.*

2. Evaluate the mean calibrator absorbance value (Cal $\bar{x}$).

Each calibrator absorbance value (after subtraction of the blank) must be greater than −0.020 and less than 0.120.

$$-0.020 < \text{Cal} < 0.120$$

If one of the calibrator absorbance values does not meet this criterion, it should be discarded and the mean value recalculated using the remaining two values. If more than one calibrator absorbance values do not meet this criterion, the run is invalid and must be repeated.

Example 1: Calculation of mean of calibrators

Calibrator well	Absorbance	Minus blank absorbance	Final calibrator absorbance
B1	0.038	0.030	0.008
C1	0.040	0.030	0.010
D1	0.039	0.030	0.009
Total absorbance			0.027

$$\text{Mean of calibrators (Cal } \bar{x}) = \frac{\text{Total absorbance}}{3} = \frac{0.027}{3} = 0.009.$$

The mean calibrator absorbance value must be greater than –0.020 and less than 0.120.

$$-0.020 < \text{Cal } \bar{x} < 0.120$$

If the mean calibrator absorbance value does not meet this criterion, the run is invalid and must be repeated.

3. Evaluate the negative control absorbance values (NC).

After subtracting the substrate blank absorbance, the absorbance values for the negative control, neutralized negative control and non-neutralized negative control must be greater than –0.020, less than 0.120 and less than the cutoff (CO) multiplied by 0.9.

$$-0.020 < \text{NC} < 0.120$$
$$\text{NC} < 0.9 \times \text{CO}$$

If the negative control absorbance values do not meet this criterion, the run is invalid and must be repeated.

4. Evaluate the positive control absorbance value (PC).

After subtracting the substrate blank absorbance, the positive control absorbance value must be greater than 0.550 and less than 1.850.

$$0.550 < \text{PC} < 1.850$$

If the positive control absorbance value does not meet this criterion, the run is invalid and must be repeated.

5. Evaluate the difference between the positive control absorbance value and the negative control absorbance value.

The difference between the positive control absorbance value and the negative control absorbance value must be greater than 0.500.

$$\text{PC} - \text{NC} > 0.500$$

If the difference between the positive control absorbance value and the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

Example 2: Calculation of difference between PC and NC

Positive control absorbance (PC) = 1.273
Negative control absorbance (NC) = 0.010
Difference (PC – NC) = 1.273 – 0.010 = 1.263

6. Calculate the percent neutralization.

The percent neutralization is calculated using the following formula:

$$\frac{\text{Absorbance for non-neutralized aliquot} - \text{Absorbance for neutralized aliquot}}{\text{Absorbance for non-neutralized aliquot} - \text{Absorbance for ETI-MAK-2 PLUS Calibrator}} \times 100$$

7. Evaluate the positive control percent neutralization.

The ETI-MAK-2 PLUS positive control must give a percent neutralization greater than 50% for the assay to be valid.

$$\text{PC Neutralization} > 50\%$$

If the percent neutralization of the positive control does not meet this criterion, the run is invalid and must be repeated.

Example 3: Calculation of positive control percent neutralization

Absorbance for non-neutralized aliquot	= 1.182
Absorbance for neutralized aliquot	= 0.334
Absorbance for ETI-MAK-2 PLUS calibrator	= 0.009

$$\frac{1.182 - 0.334}{1.182 - 0.009} \times 100 = 72.29\% \quad \text{Valid run.}$$

12. QUALITY CONTROL PROBLEM SOLVING

It is important to follow the assay procedure precisely. If calibrator or control values are not within acceptable limits (see Section 11, Quality Control) or results differ markedly from those expected, check these assay variables:

- Check incubator, incubation times, and temperatures.
- A properly functioning washer is critical to the assay. Ensure that the washer is filling and aspirating all wells, that no probes are plugged, and that the probes are placed correctly in the microwells. No fluid should be left in the wells at the end of the wash step.
- Be sure that wells do not dry out between washing and addition of the next reagent. Add reagent within a few minutes of removal of the plate from the washer. If a probe (or probes) on the washer becomes plugged during washing, identify the affected well(s) but continue with the assay procedure. Retest the affected specimen(s). To unplug probes, refer to the washer operator's manual.
- Check that all reagents and specimens are at room temperature (20-25°C) before starting the assay.
- Check that all reagents are within the expiration date, that appropriate assay kit components and ancillaries are used, and that there are no visible signs of contamination such as cloudiness or precipitates.
- Avoid cross-contamination of reagents and wells. If multichannel pipette tips have been contaminated, replace the tips.

13. INTERPRETATION OF RESULTS

Always validate quality control before evaluating results (see Section 11, Quality Control).

The presence of HBsAg in a patient sample is confirmed by comparing the absorbance value of the neutralized aliquot of a specimen to that of the non-neutralized aliquot.

Calculation of Cutoff Value

The cutoff value is determined for each plate based on the absorbance values of the calibrators run on that plate. Be sure to compare the absorbance value of each patient sample with the cutoff value computed for the plate containing that sample.

The cutoff value is determined by adding 0.040 to the mean absorbance of the calibrator values after subtraction of the substrate blank.

$$\text{CUTOFF} = \text{Cal } \bar{x} + 0.040$$

Example 4: Calculation of cutoff value

Mean absorbance for ETI-MAK-2 PLUS calibrator	0.009
Constant	+ 0.040
Cutoff value for this run	= 0.049

Calculation of Percent Neutralization

Sample acceptance criterion: absorbance of the non-neutralized aliquot (mixed with Specimen Diluent) must be greater than 0.9 x cutoff.

If the sample meets this criterion, the sample is acceptable and percent neutralization can be calculated using the formula described in Section 11.6.

Interpretation of Results for Specimens with Absorbance Within the Reading Range

Absorbance (non-neutralized aliquot)	% Neutralization	Interpretation of results
≤ 0.9 x cutoff	Any value	Not confirmed (HBsAg-negative specimen).
> 0.9 x cutoff	≤ 35%	Not confirmed (HBsAg-negative specimen).
> 0.9 x cutoff	> 35%	Confirmed (HBsAg-positive specimen).

Interpretation of Results for Specimens with Absorbance Above the Reading Range

Absorbance (non-neutralized aliquot)	% Neutralization	Interpretation of results
≤ 0.9 x cutoff	Any value	Dilute less than 1:101 (e.g., 1:51) and retest.
> 0.9 x cutoff	≤ 35%	Not confirmed (HBsAg-negative specimen).
> 0.9 x cutoff	> 35%	Confirmed (HBsAg-positive specimen).
> reading range	Any value	Dilute more than 1:101 (e.g., 1:200, 1:400, 1:800 or greater) and retest.

Example 5: Interpretation of results

Absorbance for ETI-MAK-2 PLUS calibrator = 0.009
Cutoff = 0.049
0.9 x Cutoff = 0.044

Sample No. 1

Absorbance for non-neutralized aliquot = 1.925
Absorbance for neutralized aliquot = 0.418

$$\frac{1.925 - 0.418}{1.925 - 0.009} \times 100 = 78.65\%$$ Positive specimen.

Sample No. 2

Absorbance for non-neutralized aliquot = 1.881
Absorbance for neutralized aliquot = 1.744

$$\frac{1.881 - 1.744}{1.881 - 0.009} \times 100 = 7.32\%$$ Not confirmed (negative specimen).

Sample No. 1 is confirmed positive for the presence of HBsAg and sample No. 2 is not confirmed (negative) for the presence of HBsAg.

14. LIMITATIONS OF THE PROCEDURE

- Refer to ETI-MAK-2 PLUS package insert for a description of the limitations of the procedure.
- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. See Section 12, Quality Control Problem Solving, for further information.

15. EXPECTED VALUES

In an expected values study, samples from 500 patients from an STD clinic were tested with the ETI-MAK-2 PLUS assay and all ETI-MAK-2 PLUS repeatedly reactive samples were confirmed by the DiaSorin HBsAg Confirmatory Test. The group was 49% (246/500) female and 51% (254/500) male with ages ranging from 17 to 83 years old. The percent confirmed HBsAg-positive by the DiaSorin HBsAg Confirmatory Test when used in conjunction with the ETI-MAK-2 PLUS was 0.2% (1/500).

16. SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Samples

The DiaSorin HBsAg Confirmatory Test was evaluated in conjunction with the ETI-MAK-2 PLUS assay according to the insert instructions, using the following samples:

- 200 non-selected, prospectively collected samples that were received into the lab for HBsAg or HBV-DNA testing.
- 500 non-selected, prospectively collected samples that were received from subjects recruited as coming into High-Risk (STD) Clinics.
- 20 HBsAg-reactive archive samples from subjects with a diagnosis of acute HBV infection. The sample selection was based on the fact that they showed the relevant HBV marker pattern by the reference assays (HBsAg-positive, anti-HBc-positive, IgM anti-HBc-positive, anti-HBs-negative) and that they have been classified as confirmed HBsAg-positive by neutralization using an FDA-approved HBsAg confirmatory test.
- 37 HBsAg-reactive archive samples from subjects with a diagnosis of chronic HBV infection. The sample selection was based on the fact that they have had HBsAg detectable for a period of at least six months and that they have been classified as confirmed HBsAg-positive by neutralization using an FDA-approved HBsAg confirmatory test.

After study completion, all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Using these classifications, the ETI-MAK-2 PLUS-negative and confirmed HBsAg-positive results were compared to a reference assay's HBsAg results. The following tables show the comparison of DiaSorin results with the reference assay's results. All DiaSorin initially reactive results were repeatedly reactive, confirmed positive by the DiaSorin HBsAg Confirmatory Test, and in concordance with the reference assay's confirmed results.

Prospective Sample Comparison - Results After Confirmation of Positives

Group	Reference Assay				Total
	–		+		
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS		
	–	+	–	+	
Chronic Infection	0	0	0	1*	1
Recovery	30	0	0	0	30
Past Infection	77	0	0	0	77
HBV Vaccine Response	147	0	0	0	147
Susceptible	442	0	0	0	442
Unknown	3	0	0	0	3
Total	699	0	0	1	700

* Confirmed by neutralization by both the DiaSorin and reference confirmatory assays.

Percent Agreement - Prospective Samples

Group	Negative Agreement			Positive Agreement		
	Percent	n/n	95% CI	Percent	n/n	95% CI
Chronic Infection	–	0/0	–	100%	1/1	2.5-100%
Recovery	100%	30/30	88.4-100%	–	0/0	–
Past Infection	100%	77/77	95.3-100%	–	0/0	–
HBV Vaccine Response	100%	147/147	97.5-100%	–	0/0	–
Susceptible	100%	442/442	99.2-100%	–	0/0	–
Unknown	100%	3/3	29.2-100%	–	0/0	–
Total	100%	699/699	99.5-100%	100%	1/1	2.5-100%

Archive Sample Comparison - Results After Confirmation of Positives

Group	Reference Assay				Total
	–		+		
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS		
	–	+	–	+	
Acute Infection	0	0	0	20	20
Chronic Infection	0	0	0	37	37
Total	0	0	0	57	57

Percent Agreement - Archive Samples

Group	Negative Agreement			Positive Agreement		
	Percent	n/n	95% CI	Percent	n/n	95% CI
Acute Infection	–	0/0	–	100%	20/20	83.2-100%
Chronic Infection	–	0/0	–	100%	37/37	90.5-100%
Total	–	0/0	–	100%	57/57	93.7-100%

Pregnant Women

Testing was performed on 700 serum samples belonging to a pregnant women population. All samples were tested for HBsAg using the DiaSorin ETI-MAK-2 PLUS and reference assays, in single determination per run. All HBsAg initially borderline and reactive samples were repeat tested in duplicate, per the insert instructions. HBsAg confirmatory testing by neutralization was performed on all repeatedly reactive samples. The results are summarized in the following table.

Pregnant Women Sample Comparison - Confirmatory Test

		Reference Assay		
	Result	Positive*	Negative	Total
ETI-MAK-2 PLUS	Positive*	2	0	2
	Negative	0	698	698
	Total	2	698	700

* Repeatedly reactive and confirmed by neutralization.

Negative Agreement = 100.0% (698/698) 95% CI = 99.5-100.0%.
Positive Agreement = 100.0% (2/2) 95% CI = 15.8-100.0%.

Coded Panel

A coded panel of specimens comprised of 160 frozen repository samples was tested at three external US laboratories, according to the insert instructions. The panel contained paired serum/plasma samples; the plasma was EDTA, heparin and citrate. The samples represented low-positive/borderline, mid-positive, high-positive (over range), and high-negative HBsAg levels. The results of HBsAg-positive samples are summarized in the following table.

Coded Panel Results - Sample Matrix Evaluation

Sample type		Serum	EDTA	Heparin	Citrate
Borderline Low-Positive (n = 40)	Mean %NT	74.07	74.23	75.68	73.93
	Std Dev	5.84	4.95	7.62	6.02
	95% CI*	73.66-74.49	73.88-74.58	75.14-76.22	73.51-74.36
	Total %CV	7.9%	6.7%	10.1%	8.1%
	Between matrix %CV: 1.1%				
	Across matrix total %CV: 8.2%				
Mid-Positive (n = 40)	Mean %NT	67.64	65.59	66.95	68.04
	Std Dev	5.55	5.31	3.88	5.39
	95% CI*	67.25-68.03	65.21-65.96	66.68-67.23	67.66-68.42
	Total %CV	8.2%	8.1%	5.8%	7.9%
	Between matrix %CV: 1.6%				
	Across matrix total %CV: 7.6%				
High-Positive (n = 40)	Mean %NT	77.96	77.80	78.76	79.88
	Std Dev	8.28	8.56	6.81	9.95
	95% CI*	77.37-78.54	77.20-78.40	78.28-79.24	79.18-80.58
	Total %CV	10.6%	11.0%	8.6%	12.5%
	Between matrix %CV: 1.2%				
	Across matrix total %CV: 10.7%				

* 95% CI = 95% Confidence Interval.

Confirmatory Reproducibility

Reproducibility of the confirmatory procedure was determined using a panel of 12 borderline samples composed of three sets of matched serum/plasma (EDTA, citrate, heparin). Each sample was pre-treated in three replicates and tested manually in one run per day for three days at each of three sites (two US laboratories and at DiaSorin). The results are summarized in the following table.

Reproducibility Panel Results

Sample ID #		Mean Abs	Mean %NT	Within-day CV%	Between-day CV%	Total CV%	Between-site CV%
BL01-s	RP01	0.072	77.86	4.1	4.2	5.4	1.4
BL02-s	RP02	0.066	74.67	5.9	5.0	6.9	5.8
BL03-s	RP03	0.066	75.85	4.2	2.5	5.6	6.2
BL01-e	RP04	0.067	74.70	5.5	2.7	6.2	1.5
BL02-e	RP05	0.064	74.90	4.5	3.9	5.1	4.0
BL03-e	RP06	0.067	75.75	4.8	4.7	7.0	2.2
BL01-h	RP07	0.073	78.37	6.5	3.9	6.8	2.0
BL02-h	RP08	0.062	77.29	4.7	4.8	6.0	4.8
BL03-h	RP09	0.070	75.32	4.7	3.1	4.7	3.5
BL01-c	RP10	0.067	76.99	5.5	8.3	9.8	1.6
BL02-c	RP11	0.061	75.71	4.8	5.5	6.2	2.6
BL03-c	RP12	0.064	74.80	7.8	5.6	8.4	4.1
	mean	0.067	76.02	5.2	4.5	6.5	3.3

s = serum; e = EDTA plasma; h = heparinized plasma; c = citrated plasma.

Substances That Do Not Interfere

As recommended by NCCLS Protocol EP7 (25), the DiaSorin HBsAg Confirmatory Test was evaluated for interference by testing the following substances. Testing was performed using matched pairs of negative donor serum and negative donor serum spiked with high-titer HBsAg samples to obtain a result near the cutoff. None of the compounds at the levels indicated were found to interfere with the clinical interpretation of the assay in serum.

Compound	Concentration	
Bilirubin	0.18 mmol/L	10 mg/dL
Hemoglobin	0.06 mmol/L	100 mg/dL
Triolein	33.9 mmol/L	3000 mg/dL

17. REFERENCES

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SYMBOLS USED WITH IVD DEVICES

Consult instructions for use.

In vitro diagnostic.

Biohazard.

Lot No.

Use by:

Catalogue number.

Temperature limitation.

Caution, consult accompanying documents.

Manufacturer.

Number of tests.

Manufactured By:

DiaSorin

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Saluggia, Italy

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Revised 02/2005

ETI-MAK-2 PLUS

Enzyme Immunoassay for the Detection
of Hepatitis B Surface Antigen (HBsAg)
in Human Serum or Plasma

Instruction Manual

Catalog No.: P001932

Manufactured By:

DiaSorin **DiaSorin S.p.A.**
Saluggia, Italy

Distributed By:

DiaSorin Inc.
Stillwater, MN, USA

Revised February 2005

Caution - Federal law restricts this device to sale by or on the order of a physician.

1. INTENDED USE

ETI-MAK-2 PLUS is an enzyme immunoassay (EIA) intended for the *in vitro* qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma (EDTA, citrate or heparin). The ETI-MAK-2 PLUS is intended for manual use and with the Biochem Immunosystems Labotech/ETI-LAB automated instrument.

Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates who are at high risk of acquiring HBV during the perinatal period. The ETI-MAK-2 PLUS assay's performance has not been established for the monitoring of HBV disease or therapy. This assay has not been FDA-approved for the screening of blood or plasma donors.

Assay performance characteristics have not been established when the ETI-MAK-2 PLUS HBsAg assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Assay performance characteristics have not been established for newborn testing.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis is an inflammatory disease of the liver that can severely damage the organ. The disease can result from non-infectious causes — such as biliary obstruction, biliary cirrhosis, Wilson's disease, drug toxicity, and drug hypersensitivity reactions — or from infectious viral and bacterial agents (1). Viral hepatitis is commonly caused by one of several viruses: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), or hepatitis D (delta) virus (HDV) in conjunction with HBV (1, 2), hepatitis E virus (HEV), and other as yet uncharacterized or partially characterized hepatitis viruses (non-A-E). Other viruses, including yellow fever virus, human cytomegalovirus, Epstein-Barr virus, rubella virus, herpes simplex virus, varicella-zoster virus, and some enteroviruses, can cause forms of hepatitis (1, 2).

Hepatitis B, also known as serum hepatitis, is endemic throughout the world (3-5). The infection is spread primarily through percutaneous contact with infected blood, e.g., sharing of needles by drug addicts or transfusion of blood products that have not been screened for HBV (1, 3, 6, 7). The virus is also found in virtually every type of human body fluid and has been known to be spread through oral and genital contact (1, 3, 6, 7). HBV can be transmitted from mother to child during delivery through contact with blood and vaginal secretions; it is not commonly transmitted transplacentally (6).

The incubation period for hepatitis B averages 90 days (range 40-180 days). Common symptoms include malaise, fever, gastroenteritis, and jaundice (icterus) (8). HBV infection can lead to a) icteric hepatitis, b) subclinical anicteric hepatitis, c) fulminant hepatitis, or d) chronic active or persistent hepatitis (1, 3). Over 90% of adult patients with hepatitis B completely recover from acute illness, approximately 1% die of fulminant hepatitis, and approximately 6 to 10% become chronic active or persistent carriers (1-3).

The complete hepatitis B virus, also called the Dane particle, is composed of an outer surface or envelope that carries the hepatitis B surface antigen (HBsAg) (9, 10). The envelope surrounds an inner core that contains the hepatitis B core antigen (HBcAg) (11-13). Inside the core is the HBV deoxyribonucleic acid (DNA) genome. Another antigen, the hepatitis B *e* antigen (HBeAg), is a viral core protein found in the bloodstream during active replication of HBV (14).

Because HBV is very difficult to isolate in cell culture, hepatitis B diagnosis has been based on detection of serologic markers. Early methods used to detect

serologic markers were immunodiffusion and counterimmunoelectrophoresis (3). Methods commonly used now include hemagglutination, immune adherence, latex agglutination, radioimmunoassay (RIA), and enzyme immunoassay (EIA) (1, 3). The EIA and RIA methods are the most widely utilized because of their high analytical sensitivity, analytical specificity, and ease of use.

When determining the stage of disease caused by HBV, the HBV serologic markers commonly tested for are HBsAg, antibody to HBsAg (anti-HBs), total antibody to HBcAg (total anti-HBc), immunoglobulin M antibody to HBcAg (IgM anti-HBc), HBeAg, and antibody to HBeAg (anti-HBe). Testing for these markers helps determine the presence of past or ongoing HBV infection, the acute or chronic stage of the disease, response to therapy, and/or the immune status of the patient (1, 15).

HBsAg is the viral component usually found in the highest concentration in the serum of HBV-infected patients (1, 6). It is a heterogeneous antigen. The principal determinant is called *a* and is common to all types of HBsAg. Other major determinants of the antigen are *d/y* and *w/r*. These determinant pairs are mutually exclusive, i.e., only the combinations *adw*, *adr*, *ayw*, and *ayr* are possible (10, 16).

Presence of HBsAg in serum may indicate a) acute HBV infection, b) chronic HBV infection, or c) asymptomatic carrier state (15, 17). The significance of HBsAg in serum is determined by evaluating it in relationship to the presence or absence of the other HBV markers and the clinical presentation and history of the patient.

3. PRINCIPLE OF THE PROCEDURE

ETI-MAK-2 PLUS uses monoclonal antibodies to hepatitis B surface antigen (HBsAg) as the basis for this enzyme immunoassay. The assay is a direct, noncompetitive test based on the use of polystyrene microwells coated with mouse monoclonal antibodies to HBsAg (directed to the *a* determinant of HBsAg). An enzyme tracer containing horseradish peroxidase-labeled sheep antibodies to HBsAg detects any captured HBsAg from the patient's specimen.

In the assay procedure, patient specimens and controls are incubated with incubation buffer in antibody-coated microwells. If HBsAg is present in a specimen or control, it binds to the antibody. Excess sample is removed by a wash step, and the enzyme tracer is then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. Excess enzyme tracer is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains HBsAg, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue color (650 nm). The blue color turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain HBsAg, the microwell will be colorless after the chromogen/substrate solution is added and will remain colorless after the stop solution is added. Color intensity, which is measured spectrophotometrically, is indicative of the presence of HBsAg. Absorbance value readings for patient specimens are compared to a cutoff value determined from the mean absorbance of the calibrator.

4. REAGENTS AND OTHER MATERIALS PROVIDED

Catalog Number	Product Description	Quantity/Volume
P001932	**ETI-MAK-2 PLUS**	192 tests
	Coated Strips Microwells coated with mouse monoclonal antibodies to HBsAg (IgG_1, κ class, directed to the *a* determinant of HBsAg).	Twenty-four 8-well strips (contained in 2 pouches)
	Enzyme Tracer Horseradish peroxidase-labeled sheep Fab to HBsAg, buffer, protein stabilizers. Preservative: 0.2% ProClin 300.	0.7 mL
	Tracer Diluent (Human) Buffer, human serum/plasma, protein stabilizers. Preservative: 0.2% ProClin 300.	Two 14.7-mL vials
	Calibrator (Human) Human serum/plasma non-reactive for HBsAg. Preservative: 0.2% ProClin 300.	3.3 mL
	Negative Control (Human) Human serum/plasma non-reactive for HBsAg. Preservative: 0.2% ProClin 300.	3.3 mL
	Positive Control (Human) Human serum/plasma reactive for HBsAg (subtypes *ad* and *ay*), protein stabilizers. Preservative: 0.2% ProClin 300.	2.5 mL
	Incubation Buffer Buffer, protein stabilizers, an inert blue dye. Preservative: 0.2% ProClin 300.	16 mL
	Wash Buffer (concentrate)* Buffer, detergents, preservatives.	Two 40-mL vials
	Chromogen/Substrate* Tetramethylbenzidine/hydrogen peroxide system.	Two 16-mL vials
	Stop Solution* 0.4N sulfuric acid. Caution: irritant.	30 mL
	Strip Sealers	48
	Plate Sealers	4
	Pouch Sealer	1

* *All lots of wash buffer concentrate, chromogen/substrate and stop solution are interchangeable between assay kits.*

5. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- The human blood source material used to prepare this product (i.e., tracer diluent, calibrator and negative control) derives from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 (AIDS) when tested by licensed screening tests, and found to be non-reactive for syphilis when tested by a serological test. The positive control is prepared from human blood source material reactive for HBsAg and non-reactive for antibodies to HCV, HIV-1 and HIV-2 when tested by licensed screening tests, and found to be non-reactive for syphilis when tested by a serological test. Because no test method can offer complete assurance that laboratory spec-

imens are pathogen-free, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, May 1999, and NCCLS Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue (18, 19, 20).

- All specimens, reagents, and controls should be handled as if capable of transmitting disease. Follow standard precautions for handling infectious agents during all procedures:
  - Do not pipette by mouth.
  - Do not eat, drink, smoke, or apply cosmetics in areas where specimens are handled.
  - Wear protective clothing such as lab coats, protective glasses, and disposable gloves when handling specimens and assay reagents. Wash hands thoroughly afterwards.
  - Perform all work with infectious materials in a designated area.
- Dispose of all specimens and used assay materials as if capable of transmitting disease:
  - Decontaminate liquid wastes, including those containing neutralized acid, either:
    (a) by autoclaving for 60 minutes at 121°C; or
    (b) by treating with a 1:10 or 1:100 dilution of household bleach (sodium hypochlorite concentration approximately 5%). The wastes should remain in contact with the sodium hypochlorite solution for 30 minutes for effective decontamination, after which they can be disposed of in the sink (18, 20). Do not autoclave solutions containing sodium hypochlorite.
  - Autoclave non-ignitable solids for 60 minutes at 121°C.
  - Incinerate disposable ignitable materials.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Use only dispensing equipment that has been calibrated to deliver accurate volumes, per the laboratory's standard procedures.
- **WARNING - Chromogen/substrate and the stop solution contain ingredients that can irritate skin and cause eye damage. Handle them with care. Avoid getting them in eyes or on skin or clothing. In case of contact with skin or eyes, immediately flush the affected area with water for 15 minutes. For eyes, obtain medical attention.**

  Reagents containing ProClin 300 may cause allergic reactions. Avoid prolonged contact with skin. Wash thoroughly after handling.

6. REAGENT PREPARATION

- Bring reagents to room temperature (20-25°C).
- The coated strips, calibrator, negative and positive controls, incubation buffer, chromogen/substrate and stop solution are provided ready to use.
  ***Note** - Use clean, plastic containers or acid-washed glassware for preparing the following solutions. A clean, dedicated dispenser is recommended for the working enzyme tracer to avoid contamination.*
- **Working enzyme tracer.** Bring reagents to room temperature (20-25°C). To prepare the working enzyme tracer, dilute the enzyme tracer 1:50 with tracer diluent (see chart below). After dilution, the working enzyme tracer can be used for one week if stored at 2-8°C.
  ***Caution** - Verify that the total volume prepared is sufficient for the number of tests included in the run. Use a clean container for each dilution and label the container with the reagent name, lot number of kit, lot number of reagent, plus the date of preparation and date of expiration of the working enzyme tracer.*

Number of Strips	Enzyme Tracer (µL)	Tracer Diluent (µL)	Total Volume (mL)
2	48	2352	2.4
4	80	3920	4.0
6	112	5488	5.6
8	144	7056	7.2
10	176	8624	8.8
12	208	10192	10.4

***Note** - Sufficient reagents are provided to allow for six runs per kit.*

- **Wash solution.** To prepare the working wash buffer, dilute the wash buffer concentrate (40 mL) to 1000 mL (1.0 L) with distilled or deionized water. If crystallization has occurred at 2-8°C, warm the wash buffer concentrate to 37°C and mix well before diluting. Water used for wash buffer dilution should be stored in a clean, non-metallic container to prevent contamination with peroxidase-inactivating substances. Record on the storage vial the expiration date and date of preparation of the working wash buffer. The working wash buffer can be stored for one week at 2-8°C.
  Smaller volume users may prepare less than 1 L of working wash buffer at a time if desired. If diluting only a portion of the wash buffer concentrate, check concentrate for crystallization. If crystallization has occurred during storage, warm the wash buffer concentrate to 37°C and mix well to eliminate crystals before removing aliquot for dilution.
  ***Note** - All lots of wash buffer concentrate are interchangeable between assay kits.*
- Working wash buffer containers should be thoroughly cleaned with 70% ethanol and thoroughly rinsed with distilled or deionized water before preparation of the next batch of working wash buffer.

7. REAGENT STORAGE AND HANDLING INSTRUCTIONS

- Store the test components in the refrigerator at 2-8°C away from intense light. Allow them to reach room temperature (20-25°C) before use. Return the test components to the refrigerator after use.
- Do not expose the test components to intense light, direct sunlight, or temperatures above 25°C. Do not freeze the kit.
- When stored as directed, test components will remain stable until expiration dates printed on their labels.
- Keep unused coated strips sealed in their pouches until time for use. Allow the pouch to reach room temperature (20-25°C) before opening it. Return any unused strips to the pouch as soon as possible; seal the pouch with the pouch sealer and refrigerate pouch at 2-8°C.
- After dilution, the working enzyme tracer can be stored for one week at 2-8°C.
- After dilution, the working wash buffer can be stored for one week at 2-8°C.

8. REAGENT INSTABILITY OR DETERIORATION

- The chromogen/substrate may have a slightly blue tinge. If the chromogen/substrate turns a darker blue, it may have become contaminated and should be discarded.
- Any reagent that contains visible particulate matter should be discarded.

9. SPECIMEN COLLECTION AND PREPARATION

- **This assay is not designed to test body fluids other than human serum or plasma. This assay is not designed for testing human cadaver fluids.**
- Specimens containing precipitate may give inconsistent test results. Do not test specimens containing particulate material, or grossly hemolyzed or lipemic specimens.
- The testing of heat-inactivated samples is not recommended.
- Each assay requires 100 µL human serum or plasma. EDTA, citrate or heparin anticoagulants have been tested and may be used with this assay. Follow manufacturer's instructions carefully when using plasma collection containers

with anticoagulants.

- Samples that are to be used fresh may be stored for up to two hours at 2-8°C in the presence of clots. Serum separated from the clot may be stored at 2-8°C up to 48 hours, but then must be frozen and stored deep-frozen (at –20°C or below) in sterile containers until use (21). If sample is stored frozen, mix thawed sample well before testing. It has been shown that up to three freeze-thaw cycles do not interfere with the assay.
- For shipping, specimens should be frozen at –20°C or below and shipped with dry ice. Temperature level during entire shipment should be no greater (warmer) than –20°C. Pack specimens in compliance with government regulations covering the transportation of etiologic agents (22).

10. MANUAL ASSAY PROCEDURE

Materials Provided

ETI-MAK-2 PLUS

- Coated Strips
- Enzyme Tracer
- Tracer Diluent (Human)
- Calibrator (Human)
- Negative Control (Human)
- Positive Control (Human)
- Incubation Buffer
- Wash Buffer (Concentrate)
- Chromogen/Substrate
- Stop Solution
- Strip Sealers
- Plate Sealers
- Pouch Sealer.

Materials Required But Not Provided

Microwell plate washer - The following instrument specifications are recommended for the kit performance:

Volume dispensed: 350-370 µL
Number of wash cycles: 5
Soak time: 30 seconds
Aspirate the last aliquot of dispensed liquid: yes.
***Note** - The volume of each microwell is approximately 400 µL. Make sure the volume of working wash buffer dispensed into each well does not cause the wells to overflow. If the wells overflow, set the washer to dispense less working wash buffer.*

Microwell plate reader - The following instrument specifications are recommended for the kit performance:

Wavelength: dual wavelength, 450 nm and 600-650 nm
Bandwidth: ≤ 10 nm
Absorbance range: 0 absorbance units to ≥ 2.5 absorbance units
Repeatability: better than or equal to 0.005 absorbance units, or 1%, whichever is greater
Linearity or accuracy: better than or equal to 0.010 absorbance units, or 2%, whichever is greater
Drift: less than 0.005 absorbance units per hour.

Incubator, 37°C ± 1°C.

***Note** - Gravity convection incubators are recommended. Forced-air incubators may cause edge effects. Do not use water baths as incubators.*

Micropipettes with disposable clean tips (50 µL and 100 µL).

***Note** - Suggested specifications for micropipetters (based on gravimetric testing) are:*
50 µL: accuracy ± 3%, precision 2%
100 µL: accuracy ± 2%, precision 1%.

Miscellaneous clean glass or plastic containers
Hazardous waste disposal materials
Disposable gloves
Distilled or deionized water
Pipetter-diluter (optional)
Multichannel pipetter (optional)
Pipette tips for multichannel pipetter (if multichannel pipetter is used)
Disposable reagent reservoirs (if multichannel pipetter is used)

Printer compatible with microwell reader.

Automated Procedure Using Biochem Immunosystems Labotech/ETI-LAB Instrument. See the Labotech (ETI-LAB) instrument Instruction Manual.

Assay Procedure

Perform all assay steps in the order given and without any delays between the steps. A cutoff value is calculated for each plate based on the absorbance values of the calibrators run on that plate. A maximum of one plate should be set up (completed through the first incubation step) at a time. If multiple plates are being run as a batch, each plate must be treated as a single entity; i.e., the calibrators, controls and patient specimens for the plate must be added and the incubation time started before moving on to the next plate. Proper instrument maintenance is critical for good assay performance. Follow the manufacturer's instructions for performing instrument warm-up, quality control, calibration and maintenance procedures on all equipment used in this assay.

Note - Steps 2-13 must be completed within five hours. Calibrator, positive and negative controls must be run with each plate of patient specimens.

1. Prepare assay reagents. Allow all test components to reach room temperature (20-25°C). Prepare working wash buffer and working enzyme tracer according to the directions given in Section 6, Reagent Preparation. Refer to the chart in Section 6 to ensure preparation of sufficient reagent volumes for the number of tests included in the run.

2. Prepare coated plate. Prepare enough microwells for the calibrators, controls and patient samples to be tested. Allow one blank well containing only chromogen/substrate and stop solution in well A1. Allow one well for each patient sample. The calibrator must be tested in triplicate and the negative and positive controls tested in singlet. Calibrators are to be placed in wells B1, C1 and D1; negative control is to be placed in well E1; positive control is to be placed in well F1 (for details, refer to the recommended plate map at the end of this section). Test calibrator and controls as you would patient specimens.
Coated strips may be separated. Avoid handling the bottoms of the microwells because scratches or marks could affect the reading of test results. Store unused strips in their original pouch, seal the pouch carefully, and refrigerate at 2-8°C.

3. Add incubation buffer. Add **50 µL** incubation buffer to all microwells (except for the blank well).

4. Add samples and controls. If sample was stored frozen, mix thawed sample well (vortex) before proceeding. Add **100 µL** of each calibrator, control or sample to its respective microwell. To avoid cross-contamination, use a clean micropipette tip to dispense each calibrator, control or specimen. Record the microwell position of each calibrator, control or patient specimen on a laboratory data sheet.
Incubation buffer is light blue in color. On addition of calibrators, controls or samples, the color will turn green or dark blue. This color change may vary from sample to sample, but it will always be visible.

5. Incubate. Cover the microwells with a plate or the strip sealer provided with this kit. Use a roller to affix the sealer or press firmly by hand around microwell and plate edges to ensure that the sealer is firmly attached over the entire strip or plate. Tap the coated plate gently to release any air bubbles trapped in the liquid making sure samples do not splash onto the sealer. Ensure that all microwells are filled equally. Incubate the microwells **for 2 hours ± 10 minutes at 37°C ± 1°C.**

6. Wash coated plate. Remove and discard the sealer. Aspirate the liquid from the microwells and wash each well five times as follows: Deliver 350-370 µL of working wash buffer to each microwell, let the wells soak for 30 seconds, and then aspirate the working wash buffer completely from each microwell. Microwell plate washers vary by manufacturer. Make sure the volume of working wash buffer dispensed into each well completely fills the well but does not cause the well to overflow.

7. Remove excess working wash buffer. Ensure that all microwells are aspirated completely before proceeding. With some washers it may be necessary to invert the microplate and tap it forcefully on a paper towel to effectively remove residual working wash buffer.

8. Add working enzyme tracer. Immediately add **100 µL** working enzyme tracer to each well (except for the blank well).

9. Incubate. Cover the microwells with a plate or the strip sealer provided with this kit. Ensure that sealer is applied correctly (see Step 5). Tap the coated plate gently to release any air bubbles trapped in the liquid. Ensure that all microwells are filled equally. Incubate the microwells **for 60 ± 5 minutes at 37°C ± 1°C.**

***Warning** - Timing of this incubation step is critical.*

10. Wash coated plate. Remove and discard the sealer. Aspirate the working enzyme tracer from the microwells and wash them as described in Steps 6 and 7.

11. Add chromogen/substrate. Immediately add **100 µL** chromogen/substrate to all microwells, including the blank well.
***Note** - The chromogen/substrate may have a slightly blue tinge. However, if it turns a darker blue, it may have become contaminated and should be discarded.*

12. Incubate. Incubate the microwells **for 30 ± 2 minutes at room temperature** (20-25°C). Avoid exposing the microwells to direct or intense light. Do not exceed the time limits of this incubation.

13. Add stop solution. Add **100 µL** stop solution to each microwell in the same order as chromogen/substrate was added.

14. Read results. Within one hour after addition of stop solution, read the absorbance values of the calibrators, negative control, positive control, and samples with the microwell reader set at 450/630 nm in the bichromatic mode. If time before reading exceeds one hour, the tests must be discarded and specimens retested. Check for and remove air bubbles before reading results. Record the absorbance value for each calibrator, control and sample.
***Note** - Blank the instrument on the blank well. The absorbance of the blank well containing only chromogen/substrate and stop solution (see Step 2 in Section 10, Manual Assay Procedure) is evaluated as described in the QC section. The value for the blank well should be recorded and subtracted from each calibrator, control and sample value before calculating mean values and cutoff, and before interpreting results.*

15. Perform assay quality control procedures. Before evaluating results, perform quality control procedures (see Section 11, Quality Control).

16. Perform equipment quality control and maintenance procedures. Proper instrument maintenance including calibration is critical for good assay performance. Follow the manufacturer's instructions for performing quality control and maintenance procedures on all equipment used in this assay.

Recommended Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	CAL1	S4										
C	CAL2	S5										
D	CAL3	S6										
E	NC	S7										
F	PC	S8										
G	S1	etc.										S89
H	S2											S90

11. QUALITY CONTROL

The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff.

The quality control material furnished is in a serum matrix. It may not adequately control the assay for plasma specimens. The user should provide alternate control material for testing of plasma matrices.

Each kit contains cutoff calibrator, positive and negative control sera. Acceptable values for these sera are found on the accompanying specification sheet. The test is invalid and should be repeated if the absorbance readings of either of the controls or the calibrator do not meet the specifications. If the test is invalid, patient results cannot be reported.

Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures.

It is recommended that the users refer to NCCLS C24-A and 42 CFR 493.1202 (c) for guidance on appropriate QC practices.

Use the following steps to validate quality control. References 23 and 24 provide guidance on quality control recommendations. Record the results on the QC Verification Worksheet provided for the assay.

Compute the mean absorbance value for the calibrator.

Always evaluate mean calibrator value and negative and positive control values for each plate when running more than one plate in a batch. Be sure to compare the absorbance value of each patient sample with the cutoff value computed for the plate containing that sample.

1. Evaluate the absorbance value of the substrate blank.

Blank the instrument on the blank well containing only chromogen/substrate and stop solution (see Step 14 in Section 10, Manual Assay Procedure). The absorbance value for the blank well must be between 0.000 and 0.150 for the assay to be valid. If the absorbance value of the substrate blank is less than 0.000 or greater than 0.150, the run must be repeated.

Note - Subtract the substrate blank absorbance value from each absorbance value before performing the following evaluations.

2. Evaluate the mean calibrator absorbance value (Cal $\bar{x}$).

Each calibrator absorbance value (after subtraction of the blank) must be greater than –0.020 and less than 0.120.

$$-0.020 < \text{Cal} < 0.120$$

If one of the calibrator absorbance values does not meet this criterion, it should be discarded and the mean value recalculated using the remaining two values. If more than one calibrator absorbance values do not meet this criterion, the run is invalid and must be repeated.

Example 1: Calculation of mean of calibrators

Calibrator well	Absorbance	Minus blank absorbance	Final calibrator absorbance
B1	0.038	0.030	0.008
C1	0.040	0.030	0.010
D1	0.039	0.030	0.009
Total absorbance			0.027

$$\text{Mean of calibrators (Cal } \bar{x}) = \frac{\text{Total absorbance}}{3} = \frac{0.027}{3} = 0.009.$$

The mean calibrator absorbance value must be greater than –0.020 and less than 0.120.

$$-0.020 < \text{Cal } \bar{x} < 0.120$$

If the mean calibrator absorbance value does not meet this criterion, the run is invalid and must be repeated.

3. Evaluate the negative control absorbance value (NC).

After subtracting the substrate blank absorbance, the negative control absorbance value must be greater than –0.020, less than 0.120 and less than the cut-off (CO) multiplied by 0.9.

$$-0.020 < \text{NC} < 0.120$$
$$\text{NC} < 0.9 \times \text{CO}$$

If the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

4. Evaluate the positive control absorbance value (PC).

After subtracting the substrate blank absorbance, the positive control absorbance value must be greater than 0.550 and less than 1.850.

$$0.550 < \text{PC} < 1.850$$

If the positive control absorbance value does not meet this criterion, the run is invalid and must be repeated.

5. Evaluate the difference between the positive control absorbance value and the negative control absorbance value.

The difference between the positive control absorbance value and the negative control absorbance value must be greater than 0.500.

$$\text{PC} - \text{NC} > 0.500$$

If the difference between the positive control absorbance value and the negative control absorbance value does not meet this criterion, the run is invalid and must be repeated.

Example 2: Calculation of difference between PC and NC

Positive control absorbance (PC) = 1.273
Negative control absorbance (NC) = 0.010
Difference (PC – NC) = 1.273 – 0.010 = 1.263

12. QUALITY CONTROL PROBLEM SOLVING

It is important to follow the assay procedure precisely. If calibrator or control values are not within acceptable limits (see Section 11, Quality Control) or results differ markedly from those expected, check these assay variables:

- Check incubator, incubation times, and temperatures.
- A properly functioning washer is critical to the assay. Ensure that the washer is filling and aspirating all wells, that no probes are plugged, and that the probes are placed correctly in the microwells. No fluid should be left in the wells at the end of the wash step.
- Be sure that wells do not dry out between washing and addition of the next reagent. Add reagent within a few minutes of removal of the plate from the

washer. If a probe (or probes) on the washer becomes plugged during washing, identify the affected microwell(s) but continue with the assay procedure. Retest the affected specimen(s). To unplug probes, refer to the washer operator's manual.

- Check that all reagents and specimens are at room temperature (20-25°C) before starting the assay.
- Check that all reagents are within the expiration date, that appropriate assay kit components and ancillaries are used, and that there are no visible signs of contamination such as cloudiness or precipitates.
- Avoid cross-contamination of reagents and wells. If multichannel pipette tips have been contaminated, replace the tips.

13. INTERPRETATION OF RESULTS

The presence or absence of HBsAg is determined by comparing the absorbance values of patient samples with a cutoff value. The cutoff value is determined for each plate based on the absorbance values of the calibrators run on that plate. Be sure to compare the absorbance value of each patient sample with the cutoff value computed for the plate containing that sample.

Calculation of Cutoff Value

The cutoff value is determined for each plate by adding 0.040 to the mean absorbance of the calibrator values after subtraction of the substrate blank.

$$\text{CUTOFF} = \text{Cal } \bar{X} + 0.040$$

Example 3: Calculation of cutoff value

Calibrator mean absorbance	0.009
Constant	+ 0.040
Cutoff value for this run	0.049

The cutoff was established by testing 348 samples (174 volunteer blood donors and 174 hospitalized patients) with three lots of ETI-MAK-2 PLUS. The results were examined as the difference (delta) between single sample absorbance and calibrator absorbance. In the apparently healthy adult (volunteer donor) population, 95% had delta values less than 0.030 and 99% had delta values less than 0.031; in the hospitalized patient population, 95% had delta values less than 0.030 and 99% had delta values less than 0.047.

Interpretation of Results (Manual or Labotech/ETI-LAB assay)

Initial Results

Absorbance Values	Result	Interpretation
Absorbance ≤ 90% x Cutoff	Not Reactive	HBsAg not detected by ETI-MAK-2 PLUS. These samples are deemed negative for HBsAg and need not be further tested. This result should not be used alone but in conjunction with other hepatitis B serological markers to determine disease state.
Absorbance > 90% x Cutoff	Initially Reactive	Initial detection of HBsAg by ETI-MAK-2 PLUS. All initially reactive samples must be retested in duplicate using the ETI-MAK-2 PLUS assay. See Interpretation of Repeat and Final Results.

Note - *The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antigen present.* There may be a specimen dilutional effect with citrated plasma due to the liquid nature of this anticoagulant. Borderline or high-negative results obtained from citrated specimens should be retested using serum as the matrix.

Example 4: Interpretation of samples

Cutoff = 0.049.
Sample No. 1 absorbance = 0.012.
Sample No. 2 absorbance = 0.908.

Sample No. 1 should be considered negative for HBsAg; sample No. 2 should be considered initially reactive for HBsAg and repeat tested.

Repeat and Final Results

Absorbance Values	Result	Interpretation
If 2 out of 3 results Absorbance ≤ 90% x Cutoff	Not Reactive	HBsAg not detected by ETI-MAK-2 PLUS. These samples are deemed negative for HBsAg and need not be further tested. This result should not be used alone but in conjunction with other hepatitis B serological markers to determine disease state.
If 2 out of 3 results Absorbance > 90% x Cutoff	Repeatedly Reactive	Repeat detection of HBsAg by ETI-MAK-2 PLUS. All repeatedly reactive samples must be confirmed using the DiaSorin HBsAg Confirmatory Test (Cat. N. N0143), which is a neutralization procedure.

Example 5: Interpretation of samples

Cutoff = 0.049.
Sample No. 1 absorbances = 0.042, 0.049.
Sample No. 2 absorbances = 0.905, 0.895.

Sample No. 1 should be considered negative for HBsAg; sample No. 2 should be considered repeatedly reactive for HBsAg and confirmed by neutralization using the DiaSorin HBsAg Confirmatory Test.

14. LIMITATIONS OF THE PROCEDURE

- Results obtained from immunosuppressed patients should be interpreted with caution.
- This assay is not designed to test body fluids other than human serum or plasma.
- Any laboratory test result should be interpreted in conjunction with the patient's clinical presentation and the results of other diagnostic tests. A negative result on a given laboratory assay does not rule out the possibility of infection.
- The prevalence of the analyte will affect the assay's predictive value.
- Assay performance characteristics have not been established when the ETI-MAK-2 PLUS HBsAg assay is used in conjunction with the other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.
- Performance characteristics have not been established for any other automated instrument than the Biochem Immunosystems Labotech/ETI-LAB automated instrument. If another automated instrument is used, the user is responsible for establishing their own assay performance characteristics.
- Specimens from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such specimens may produce false negative results when tested with a direct, non-competitive immunoassay containing a monoclonal antibody such as the ETI-MAK-2 PLUS assay. Specimens from these individuals should not be tested with this assay.
- The analytical sensitivity of the ETI-MAK-2 PLUS has been determined to be approximately 0.1 PEI U/mL.

15. EXPECTED VALUES

The 436 prospective samples used in the expected values study for the ETI-MAK-2 PLUS assay were from patients who were sent to the laboratory for HBV testing. Of those samples, 100 (23%) were frozen and 336 (77%) were fresh. The patients represented Florida, Georgia, Pennsylvania, California, Utah, and the Southeastern US. The group was 71% (309/436) female, 28% (121/436) male and 1% (6/436) unspecified; the ethnicity of the patients was unspecified. The ages ranged from 1 to 89 years old, with six samples unspecified. The percent ETI-MAK-2 PLUS initially reactive results in these samples was 5%.

The table below summarizes the percent ETI-MAK-2 PLUS initially reactive and negative results by gender and age range. There were six samples for which gender and age were not reported; they were all positive. There were six other samples for which age was not reported, two were from females and four were from males; all were negative. These 12 results were not included in the table.

		ETI-MAK-2 PLUS				
		Initially Reactive*		Negative		
Age Range	Gender	n	%	n	%	Total
0-9	F	0	0%	2	100%	2
	M	0	0%	1	100%	1
10-19	F	1	4%	27	96%	28
	M	1	33%	2	67%	3
20-29	F	3	3%	111	97%	114
	M	4	19%	17	81%	21
30-39	F	1	1%	89	99%	90
	M	3	8%	34	92%	37
40-49	F	4	11%	31	89%	35
	M	2	8%	23	92%	25
50-59	F	1	8%	12	92%	13
	M	1	7%	13	93%	14
60-69	F	1	20%	4	80%	5
	M	0	0%	5	100%	5
70-79	F	1	7%	13	93%	14
	M	0	0%	5	100%	5
80-89	F	1	17%	5	83%	6
	M	0	0%	6	100%	6
Total		24	6%	400	94%	424

* Initially reactive; not repeat tested or confirmation performed.

High-Risk Population. Single repository samples belonging to high-risk populations (66 hemodialyzed patients, 148 hemophiliacs, 150 IV drug users) and 500 prospectively collected samples from STD clinic patients were tested with the ETI-MAK-2 PLUS assay to determine frequency of positive results in that population. The group was 33% (288/864) female, 59% (506/864) male, and 8% (70/864) unspecified, with ages ranging from 17 to 87 years old. No geographical locations were specified. Equivocal results by the ETI-MAK-2 PLUS assay were repeated if sample volumes permitted. The table below summarizes the ETI-MAK-2 PLUS initial results. The data in the table represent the number of specimens in each group.

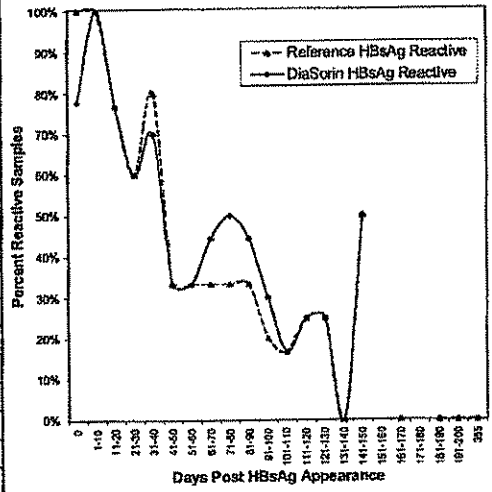
High-Risk Population	Frequency of Initially Reactive Results (# Positive/Total # Samples)
IV Drug Users	19/150 = 12.7%
Hemophiliacs	12/148 = 8.1%
Hemodialysis Patients	0/66 = 0.0%
STD Clinic Patients	1/500 = 0.2%
Total	32/864 = 3.7%

Acute Serial Panels. One hundred twenty-four (124) archived serial samples from nine individuals were analyzed. Most (8/9) of these individuals were defined as being acutely infected by the appearance of HBsAg and HBeAg with the subsequent appearance of IgM anti-HBc, total anti-HBc, anti-HBe, and anti-HBs. One individual had detectable HBsAg, but did not have detectable HBeAg in any specimen. However, this individual did seroconvert for anti-HBe.

The specimens were collected from individuals undergoing plasmapheresis for further manufacturing purposes. Three individuals were found to be infected with HBV during the first plasmapheresis and others became infected with HBV during subsequent plasmaphereses. It is unknown how long these three initially HBsAg-reactives were infected prior to the first plasmapheresis. All nine individuals underwent sequential plasmaphereses after becoming HBV-infected. However, the timing of subsequent plasmaphereses varied from individual to individual. The specimens draw times were normalized to represent the day that HBsAg was first detected by an FDA-licensed assay as day 0. Draw days ranged from day 0 (HBsAg first detected) through day 355 post-day 0. Since all panels did not contain the same draw day, sample results were grouped within day intervals (e.g., days 0, 1-10, 11-20, etc., representing days since first detection of HBsAg).

The results are summarized in the following table and graph. In the graph below the pattern for the reference HBsAg percent reactive has been overlaid for reference.

Day Range	Number of Specimens	DiaSorin HBsAg Reactive	Percent Positive
0	9	7	77.8
1-10	10	10	100
11-20	13	10	76.9
21-30	9	6	66.7
31-40	10	7	70.0
41-50	6	2	33.3
51-60	10	3	30.0
61-70	9	4	44.4
71-80	6	3	50.0
81-90	9	4	44.4
91-100	10	3	30.0
101-110	6	1	16.7
111-120	4	1	25.0
121-130	4	1	25.0
131-140	3	0	0.0
141-150	2	1	50.0
151-160	0	0	NA
161-170	1	0	0.0
171-180	0	0	NA
181-190	1	0	0.0
191-200	1	0	0.0
355	1	0	0.0



16. PERFORMANCE DATA

Clinical Samples

Prospective Samples. A study of 836 prospective specimens was conducted. These specimens represented individuals who were sent to the laboratory for hepatitis testing or represented a high-risk population. Specimens were collected at a reference laboratory or an STD clinic and assayed at the California clinical trial site. The patients were 61% (510/836) female and 39% (326/836) male. The ages ranged from 1 to 89 years old, with three specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-MAK-2 PLUS procedure according to the assay procedure, including confirmation by neutralization of repeatedly reactive samples using the DiaSorin HBsAg Confirmatory Test. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure, including confirmation by neutralization of repeatably reactive HBsAg samples by the reference assay's confirmatory procedure.

Results by Specimen Classification. After study completion, all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 11 unique HBV marker patterns observed in the ETI-MAK-2 PLUS prospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Chronic Infection	+	–	–	+	+	–	1
	+	–	–	+	–	–	1
Recovery	–	–	+	+	–	–	2
	–	–	+	+	–	+	2
	–	–	–	+	+	+	19
	–	–	–	+	+	–	9
Past Infection	–	–	–	+	–	+	43
	–	–	–	+	–	–	42
HBV Vaccine Response	–	–	–	–	–	+	185
Not Previously Infected with HBV	–	–	–	–	–	–	529
Unknown	–	–	–	–	–	I	3

I = Indeterminate result.

Based on the above classifications, the ETI-MAK-2 PLUS HBsAg results for the prospective samples were compared to a reference assay's HBsAg results. The following table shows this comparison and percent agreement with 95% exact confidence intervals with the reference HBsAg assay results.

Prospective Samples Comparison

Reference Serology Classification	Reference HBsAg Assay			TOTAL
	–		+[a]	
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS	
	–	+	+	
Chronic Infection	0	0	2[b]	2
Recovery	31	0	0	31
Past Infection	86	0	0	86
HBV Vaccine Response	185	0	0	185
Not Previously Infected with HBV	528	1[b]	0	529
Unknown	3	0	0	3
Total	833	1	2	836

[a] Repeatedly reactive and confirmed by HBsAg Confirmatory Test.
[b] DiaSorin HBsAg Confirmatory Test was not performed on one sample.

Prospective Samples

Chronic Infection	Positive agreement with reference assay results =	100% (2/2)[a]
	95% CI =	15.8-100%
	Negative agreement with reference assay results =	NA (0/0)
	95% CI =	NA
Recovery	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (31/31)
	95% CI =	88.8-100%
Past Infection	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (86/86)
	95% CI =	95.8-100%
HBV Vaccine Response	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (185/185)
	95% CI =	98.0-100%
Not Previously Infected with HBV	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	99.8% (528/529)[a]
	95% CI =	99.0-99.9%
Unknown	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (3/3)
	95% CI =	29.0-100%

[a] DiaSorin HBsAg Confirmatory Test was not performed on one sample.

Retrospective Samples. Retrospective studies were carried out at three clinical laboratories in the United States (California, Missouri, and Minnesota) and at DiaSorin (Italy) to assess the performance of the ETI-MAK-2 PLUS assay in detecting HBsAg. The study set included 650 frozen repository samples (the majority of which were purchased from commercial vendors) from the following populations:

- patients with chronic hepatitis B infection (HBsAg-positive for greater than six months) - 111 frozen repository samples;
- patients with serologically diagnosed hepatitis B infection (acute, chronic, asymptomatic, convalescent, etc.) - 82 frozen repository samples;
- patients sent to the laboratory for hepatitis B testing - 100 frozen repository samples;
- a general hospital patient population - 357 frozen repository samples.

The specimens represented Midwestern (2%), Southeastern (25%), Western (13%), and Northeastern US (2%), outside of the US (1%), and unspecified (57%). The group was 44% (287/650) female, 42% (270/650) male, and 14% (93/650) unspecified. Approximately 13% (84/650) were Caucasian, 4% (27/650) were African American, < 1% (5/650) were Hispanic, < 1% (3/650) were Asian, and 82% (531/650) were unspecified. The ages ranged from 5 to 98 years old, with 131 specimens not specified.

The study (testing) sites were blinded to the previous specimen categorization. All testing was performed by the manual ETI-MAK-2 PLUS procedure. Specimens were characterized by testing with six HBV serological markers (HBsAg, HBeAg, IgM anti-HBc, total anti-HBc, anti-HBe, anti-HBs) using FDA-licensed or approved assays. Testing with these assays followed the FDA-licensed or approved procedure with the exception of the HBsAg assay at two of the three sites. At these sites, the majority of specimens that were initially HBsAg-positive were repeated in duplicate; however, the repeatedly reactive specimens were not confirmed by the licensed HBsAg confirmation assay at the two sites. Therefore, true HBsAg result was determined in one of three ways: 1) confirmed by reference assay neutralization during clinical trials, 2) based on a statement by the attending physician that HBsAg was positive for greater than six months, or 3) information provided by the vendor regarding confirmatory testing performed at their location or by the material source facility. Ten samples were excluded from analysis (four hospital patients and six patients who

came to the lab for HBV testing), because the true HBsAg result for these samples was not determined by one of these three methods.

Results by Specimen Classification. After study completion, all samples were assigned a specimen classification based on the patterns of the six HBV serological markers established by the reference assays. Based on these serological marker patterns, the samples were categorized into the HBV classifications described in the table below. There were 35 unique HBV marker patterns observed in the ETI-MAK-2 PLUS retrospective clinical studies.

Characterization Based On Single Point Specimen	HBsAg	HBeAg	IgM anti-HBc	Total anti-HBc	anti-HBe	anti-HBs	n
Acute Infection	+	+	+ or I	+	–	–	52
	+	–	+ or I	+	+	–	4
	+	–	–	–	–	–	2
	+	+	–	–	–	–	2
Chronic Infection	+	–	–	+	+	–	82
	+	+	–	+	–	–	21
	+	–	–	+	– or I	–	23
	+	+	+	+	–	+	4
	+	+	– or I	+	–	+	2
	+	–	–	+	+	+	2
	+	+	–	+	+ or I	+	2
	+	+	+	+	+	+	1
	+	+	–	+	+	–	1
	+	–	–	+	–	+	1
Recovery	–	–	–	+	+ or I	+	40
	–	–	–	+	+	–	6
	–	–	+	+	+	–	2
	–	–	+ or I	+	+	+	2
Past Infection	–	– or I	–	+	–	+	12
	–	–	–	+	–	–	9
HBV Vaccine Response	–	–	–	–	–	+	20
Not Previously Infected with HBV	–	–	–	–	–	–	343
Uninterpretable	–	+ or I	–	–	–	–	13
	–	+	–	+	–	+	2
	–	+	–	+	+	+	1
	–	I	–	+	–	–	1

I = Indeterminate result.

Based on the above classifications, the ETI-MAK-2 PLUS HBsAg results for the retrospective samples were compared to a reference assay's HBsAg results. The following tables show this comparison and percent agreement with 95% exact confidence intervals with the reference HBsAg assay results. The data are presented in three tables based on the means of determining true HBsAg results.

Retrospective Samples Comparison:
True HBsAg Result Based on Statement from Physician

Reference Serology Classification	Reference HBsAg Assay		TOTAL
	–	+	
	ETI-MAK-2 PLUS	ETI-MAK-2 PLUS	
	–	+	
Acute Infection	0	26	26
Chronic Infection	0	61	61
Recovery	1	0	1
Not Previously Infected with HBV	1	0	1
Total	2	87	89

Acute Infection
Positive agreement with reference assay results = 100% (26/26)
95% CI = 86.8-100%
Negative agreement with reference assay results = NA (0/0)
95% CI = NA

Chronic Infection
Positive agreement with reference assay results = 100% (61/61)
95% CI = 94.1-100%
Negative agreement with reference assay results = NA (0/0)
95% CI = NA

Recovery
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (1/1)
95% CI = 2.5-100%

Not Previously Infected with HBV
Positive agreement with reference assay results = NA (0/0)
95% CI = NA
Negative agreement with reference assay results = 100% (1/1)
95% CI = 2.5-100%

Retrospective Samples Comparison:
True HBsAg Result Based on Confirmation by Neutralization During Clinical Trials

Reference Serology Classification	Reference HBsAg Assay				TOTAL
	–		+		
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS		
	–	+	–	+	
Acute Infection	0	0	0	25	25
Chronic Infection	0	0	1	47	48
Recovery	16	0	0	0	16
Past Infection	6	0	0	0	6
HBV Vaccine Response	6	0	0	0	6
Not Previously Infected with HBV	110	3	0	0	113
Uninterpretable	5	2	0	0	7
Total	143	5	1	72	221

Acute Infection	Positive agreement with reference assay results =	100% (25/25)
	95% CI =	86.3-100%
	Negative agreement with reference assay results =	NA (0/0)
	95% CI =	NA
Chronic Infection	Positive agreement with reference assay results =	97.9% (47/48)
	95% CI =	88.9-99.9%
	Negative agreement with reference assay results =	NA (0/0)
	95% CI =	NA
Recovery	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (16/16)
	95% CI =	79.4-100%
Past Infection	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (6/6)
	95% CI =	54.1-100%
HBV Vaccine Response	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (6/6)
	95% CI =	54.1-100%
Not Previously Infected with HBV	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	97.3% (110/113)
	95% CI =	92.4-99.4%
Uninterpretable	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	71.4% (5/7)
	95% CI =	29.0-96.3%

Retrospective Samples Comparison:
True HBsAg Result Based on Vendor Information

Reference Serology Classification	Reference HBsAg Assay			TOTAL
	–		+	
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS	
	–	+	+	
Acute Infection	0	0	6	6
Chronic Infection	0	0	23	23
Recovery	33	0	0	33
Past Infection	15	0	0	15
HBV Vaccine Response	13	1	0	14
Not Previously Infected with HBV	222	7	0	229
Uninterpretable	9	1	0	10
Total	292	9	29	330

Acute Infection	Positive agreement with reference assay results =	100% (6/6)
	95% CI =	54.1-100%
	Negative agreement with reference assay results =	NA (0/0)
	95% CI =	NA
Chronic Infection	Positive agreement with reference assay results =	100% (23/23)
	95% CI =	85.2-100%
	Negative agreement with reference assay results =	NA (0/0)
	95% CI =	NA
Recovery	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (33/33)
	95% CI =	89.4-100%
Past Infection	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	100% (15/15)
	95% CI =	78.2-100%
HBV Vaccine Response	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	92.9% (13/14)
	95% CI =	66.1-99.8%
Not Previously Infected with HBV	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	96.9% (222/229)
	95% CI =	93.8-98.8%
Uninterpretable	Positive agreement with reference assay results =	NA (0/0)
	95% CI =	NA
	Negative agreement with reference assay results =	90.0% (9/10)
	95% CI =	55.5-99.7%

Samples from Pregnant Women. Single samples prospectively collected from pregnant women (n = 700) were tested with both DiaSorin and reference HBsAg assays according to the insert procedures. All HBsAg repeatedly reactive samples were confirmed by neutralization using the DiaSorin and reference assay's confirmatory tests. All HBsAg confirmed positive samples were verified by testing for HBeAg, anti-HBe, total anti-HBc, IgM anti-HBc and anti-HBs at the trial site. The table below compares the negative and confirmed positive results by the ETI-MAK-2 PLUS assay and the HBsAg reference assay. The data in the table represent the number of specimens in each group. Equivocal results by the ETI-MAK-2 PLUS were repeated per the insert instructions, if sample volumes permitted, and the repeat results used in the calculation.

Pregnant Women Samples Comparison

Group	Reference HBsAg Assay			TOTAL
	-		+	
	ETI-MAK-2 PLUS		ETI-MAK-2 PLUS	
	-	Repeatedly Reactive	+	
Prospective Samples	695	3[a]	2[b]	700

[a] Repeatedly reactive but not confirmed by DiaSorin HBsAg Confirmatory Test.
[b] Repeatedly reactive and confirmed by DiaSorin HBsAg Confirmatory Test.

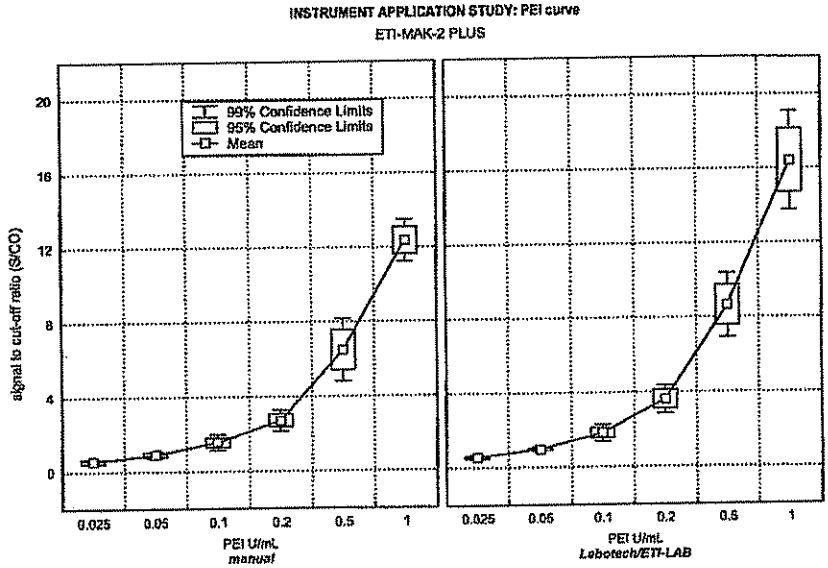
Pregnant Women Samples

Percent Positive Agreement	= 100% (2/2)	95% CI = 15.8-100%
Percent Negative Agreement	= 100% (698/698)	95% CI = 99.5-100%

Instrument Comparison of Biochem Immunosystems Labotech/ETI-LAB to the Manual Method

An instrument application study was conducted at DiaSorin, Saluggia (Italy), to evaluate the performance of the ETI-MAK-2 PLUS assay on the Biochem Immunosystems Labotech/ETI-LAB, an automated microplate processing instrument, compared to the manual analysis. The Paul-Ehrlich-Institut (PEI) Standard, 12 serum samples near the ETI-MAK-2 PLUS cutoff and samples from the clinical trials (32 suspected hepatitis B patients and eight apparently healthy adults) were tested in parallel manually and on the Labotech.

Serial dilutions of the PEI Standard were prepared in fetal calf serum to obtain a panel ranging from high concentration to below the analytical sensitivity of the assay. The diluted Standard samples were tested in duplicate, one run per day for three days both manually and on the Labotech. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The 95% confidence intervals were established for the S/CO values of each point of the Standard-referenced curve and therefore the analytical endpoint sensitivity was defined (first dilution with S/CO > 1.1). A graph summarizing these results is presented below.



The 12 samples near the cutoff were tested in triplicate, one run per day for three days both manually and on the Labotech. The samples from the clinical trials were tested in singlet in one run on one day, both manually and on Labotech. The mean, the standard deviation and the coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each of the tested specimens. A summary of the data is presented in the following table.

	Manual			Labotech/ETI-LAB		
Analytical Endpoint Sensitivity (0.1 PEI U/mL)	Mean	W/R* %CV	D/D* %CV	Mean	W/R %CV	D/D %CV
S/CO [95% CI]*	1.56 [1.29-1.82]	11.8	15.1	1.84 [1.56-2.12]	4.1	15.7
12 Cutoff Samples S/CO Range of mean S/CO	1.21 0.91-1.44	11.2	7.9	1.43 1.11-1.80	4.0	13.0
Clinical Samples						
Suspected Hepatitis B Range of S/CO	Negative: NA (0/32) Equivocal: NA (0/32) Positive: 23.9- > 39 (32/32)			Negative: NA (0/32) Equivocal: NA (0/32) Positive: 35.5- > 62 (32/32)		
Healthy Adults Range of S/CO	Negative: 0.24-0.64 (8/8) Equivocal: NA (0/8) Positive: NA (0/8)			Negative: 0.08-0.46 (8/8) Equivocal: NA (0/8) Positive: NA (0/8)		

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.
* 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day.

Reproducibility

Manual Assay. Intra-assay, inter-assay, inter-lot, and inter-site variability studies were carried out on the ETI-MAK-2 PLUS kit to test the variability within runs, between runs, between days, between kit lots, and between test sites. Variability was measured on a panel of ten sera that included negative, borderline, and positive samples. Three ETI-MAK-2 PLUS kit lots were tested at three independent test sites. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The results of that study are tabulated below.

Clinical Site Reproducibility Study

ID#	Class.	# of Tests per Sample	Mean S/CO	Within-run %CV	Between-run %CV	Between-lot %CV	Between-day %CV	Between-site %CV	Total %CV
S01	High	108	13.09	6.08	12.11	10.63	9.70	9.03	16.51
S02	High	108	5.59	4.44	10.89	7.82	11.60	8.98	17.50
S03	Low	108	3.19	4.51	11.45	12.04	7.76	9.33	18.66
S04	Low	108	2.70	7.47	8.14	12.65	8.81	6.72	20.18
S05	Low	108	3.67	7.81	8.17	7.24	9.71	8.04	14.83
S06	Low	108	2.57	4.40	17.13	12.58	16.81	7.31	25.88
S07	B-line	108	1.33	5.37	13.09	10.89	14.11	15.62	29.05
S08	B-line	108	0.96	9.19	10.09	13.48	8.82	5.00	24.04
S09	B-line	108	1.01	15.66	13.89	16.93	20.65	3.66	32.00
S10	Neg	108	0.22	15.71	29.33	30.67	59.12	21.18	104.13

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.

Instrument Reproducibility. An instrument reproducibility study was conducted at two external sites (Georgia, Massachusetts) and at DiaSorin (Italy) to evaluate the performance of the ETI-MAK-2 PLUS assay on the Biochem Immunosystems Labotech/ETI-LAB (an automated microplate processing instrument). Twelve samples were used in the study: eight low-positive and four high-negative samples. The samples were prepared by spiking high-positive sera into each of 12 negative serum samples to reach the various analyte levels. All samples were tested in triplicate in each run, two runs per day for five days. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The mean, standard deviation and coefficient of variation (CV%) of the S/CO values were computed by the

different components of variability for each instrument and across instruments. A summary of the data is presented in the following table.

Sample ID#	# of Tests	Mean S/CO	Within-run %CV	Between-run %CV	Between-day %CV	Between-instrument %CV	Total %CV
LP1	90	2.02	8.1	7.8	8.4	5.3	14.6
LP2	90	1.85	2.8	8.0	9.3	8.8	11.2
LP3	90	1.97	5.7	6.8	3.6	4.3	10.4
LP4	90	1.36	3.0	6.6	5.8	2.1	9.9
LP5	90	2.28	5.2	6.2	8.8	7.5	14.2
LP6	90	1.74	3.1	3.2	10.0	8.4	15.7
LP7	90	1.81	3.2	7.0	12.1	8.7	12.8
LP8	90	1.90	3.7	6.8	11.6	6.7	13.7
HN1	90	0.82	3.2	5.8	8.7	5.1	11.8
HN2	90	0.91	6.9	8.3	5.4	2.1	11.0
HN3	90	0.71	5.7	8.3	11.0	9.1	14.8
HN4	90	0.85	8.1	9.8	12.7	6.2	15.1
Low pos. mean		1.87	3.4	6.8	9.0	7.1	13.2
High neg. mean		0.82	6.3	8.3	9.8	5.6	13.2

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.
LP = Low Positive; HN = High Negative.

Plasma reproducibility on the Labotech has not been established. If plasma is used on the Labotech, the user should establish appropriate assay reproducibility in accordance with NCCLS EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices.

Plasma Reproducibility. A plasma reproducibility study was conducted at DiaSorin, Saluggia (Italy), to evaluate the performance of the manual ETI-MAK-2 PLUS assay on serum versus a variety of plasma types. The plasma types evaluated were citrate, heparin and EDTA. Sample sets of matched serum/multiple plasma were used in the study. A sample set was prepared by spiking the same high-positive sample into each of the matrices (serum and plasmas) resulting in a total of four specimens per set around the cutoff. Several high-positive samples were used in the preparation of the 12 different near-cutoff sample sets. Six matched serum/multiple plasma sample sets were tested in triplicate in each run; thus there were two runs per day for three days, all tested in a manual mode. Due to the requirement that assay cutoff be established for each plate, reproducibility was evaluated based on specimen absorbance-to-cutoff ratios (S/CO) rather than absolute absorbance values. The mean, the standard deviation and the coefficient of variation (CV%) of the S/CO values were computed by the different components of variability for each of the tested specimens. The 95% confidence intervals were established for the S/CO values of all serum samples and each plasma type. A summary of the data is presented in the following table.

	Serum	Citrate	Heparin	EDTA
Mean S/CO	0.91	0.92	0.87	0.91
95% CI*	0.87-0.94	0.88-0.96	0.83-0.91	0.88-0.95
W/R* %CV	6.3%	6.9%	7.1%	7.8%
D/D* %CV	8.1%	9.6%	6.7%	8.0%
Total %CV	9.9%	11.6%	9.6%	10.0%
Between matrix %CV: 8.5%				
Across matrix total %CV: 13.1%				

%CVs were calculated using specimen absorbance-to-cutoff ratios (S/CO) which normalized the data plate-to-plate.
* 95% CI = 95% Confidence Interval; W/R = within-run; D/D = day-to-day.

Analytical Sensitivity

The analytical sensitivity of the assay (the smallest quantity of analyte that can be distinguished from background) was evaluated using single-point serial dilutions of a standard preparation from the Paul-Ehrlich-Institut (PEI). The analytical sensitivity of the assay (last positive dilution) was determined to be 0.1 PEI U/mL (Mean Signal-to-Cutoff Ratio = 1.56; 95% Confidence Interval = 1.29-1.82).

Cross-Reactivity

Of the 525 potentially interfering samples, 477 (91%) were negative and 48 (9%) were positive by ETI-MAK-2 PLUS. Among the 48 positive samples, 12 were negative for HBeAg and IgM anti-HBc and negative for HBsAg on repeat testing; 36 were positive by reference testing or review of hepatitis B marker patterns for those samples. As expected, individuals infected by HDV are also infected with HBV. Disease was determined by serological testing; there is no guarantee that the associated antigen was present in the tested material. Interference testing with the described specimens was not performed.

Cross-Reactivity Study Results

Group	n	ETI-MAK-2 PLUS Negative Samples	ETI-MAK-2 PLUS Positive Samples	% Confirmed Positive By Additional Testing
Acute EBV Infection	16	13	3[a]	–
Acute CMV Infection	20	17	3[a]	–
Acute HSV Infection	10	10	0	–
Acute Toxoplasmosis	18	18	0	–
Acute Parvovirus B19 Infection	5	5	0	–
HTLV-I/II Infection	50	47	3[b]	100% (1/1)
Syphilis	26	25	1[a]	–
HCV Infection	50	48	2[a]	–
HDV Infection	20	1	19	100% (19/19)
HIV Infection	50	50	0	–
Acute HAV Infection	50	47	3	100% (3/3)
Past HAV Infection	50	44	6[c]	100% (5/5)
Rheumatoid Factor (RF) +	40	40	0	–
Autoimmune Disease, Including SLE	30	30	0	–
Autoimmune Hepatitis	5	5	0	–
Myeloma	20	20	0	–
Hypergammaglobulinemia	20	20	0	–
Influenza Vaccine	5	5	0	–
Elevated Liver Enzymes	10	9	1	100% (1/1)
Non-viral Liver Disease	30	23	7	100% (7/7)
TOTAL	525	477 (91%)	48 (9%)	100% (36/36)

[a] These samples were negative for HBeAg and IgM anti-HBc, and negative on repeat testing on ETI-MAK-2 PLUS.
[b] Two samples were negative for HBeAg and IgM anti-HBc, and negative on repeat testing on ETI-MAK-2 PLUS.
[c] One sample was negative for HBeAg and IgM anti-HBc, and negative on repeat testing on ETI-MAK-2 PLUS; five samples were positive by reference testing.

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SYMBOLS USED WITH IVD DEVICES

Consult instructions for use.

In vitro diagnostic.

Biohazard.

Lot No.

Use by:

Catalogue number.

Temperature limitation.

Caution, consult accompanying documents.

Manufacturer.

Number of tests.

Manufactured By:

DiaSorin

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