Aware™ BED™ EIA
HIV-1 Incidence Test
(IgG-Capture HIV-EIA)

Enzyme Immunoassay for
Population Estimates of HIV-1 Incidence

Cat. No. 98003

Includes: 192 tests, Two Packs
98003A - Refrigerator Pack - Store at 2-8° C
98003B - Freezer Pack - Store at ≤-20° C

FOR SURVEILLANCE USE ONLY
Not for diagnostic or clinical use

Manufactured in the USA by:
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NAME AND INTENDED USE
The Aware™ BED™ EIA HIV-1 Incidence Test is an in vitro quantitative enzyme immunoassay for the determination of the proportion of HIV-1-specific IgG in blood samples, including serum, plasma, and dried blood, serum, and plasma spots [12] with respect to total IgG as an aid in determining the elapsed time since an HIV-1 infection occurred. The Aware™ BED™ EIA is to be used for surveillance purposes only, such as for population incidence estimates to assist in prevention programs, targeting resources, monitoring and evaluation, and identifying high-risk cohorts for prevention research, including vaccine trials. The assay is not for clinical use or for use in the diagnosis of HIV infection.

SUMMARY AND EXPLANATION OF THE TEST
Since the development of the first less sensitive EIA to detect recent HIV-1 seroconversion [1], there has been a growing interest in the application of laboratory methods to measure HIV-1 incidence in various cross-sectional populations [2]. The measurement of HIV-1 incidence is important for monitoring the effectiveness of prevention programs, targeting resources and second-generation surveillance. Currently, a modification of another commercially available EIA is used by some U.S. laboratories to measure HIV-1 incidence [3]. However, less sensitive EIAs are useful in populations with mainly subtype B infections [4,5]. The Aware™ BED™ EIA is a second-generation assay to detect recent HIV-1 seroconversion [6] and was developed to address some of the shortcomings (e.g. 1:20,000 dilution, assay variability, subtype-dependent performance) of the less sensitive EIAs [4,5]. The Aware™ BED™ EIA has been used in a number of cross-sectional populations to estimate incidence and evaluate association with various risk factors [7, 8, 14, 15, 16, 17, 18]. The Aware™ BED™ EIA referenced in this Package Insert was developed and initially manufactured and distributed by the United States Centers for Disease Control and Prevention (CDC). Calypte® Biomedical Corporation has licensed the technology from the CDC and had been manufacturing and distributing the test as the “Calypte® HIV-1 BED Incidence EIA” since 2004. The product name was changed to “Aware™ BED™ EIA” in 2008.

GENERAL KIT INFORMATION
The Aware™ BED™ EIA is a quantitative antibody assay. A threshold cutoff based on a calibrator specimen determines the classification of recent seroconversion. Therefore, the assay protocol must be strictly adhered to for accurate, precise, and reproducible results. The kit contains two 96-well plates with twelve (12) 1x8 removable strips and all necessary reagents, including controls and calibrator. Each plate assays 11 controls plus 85 specimens in the initial testing mode. Specimens that are initially reactive in the assay are repeat tested in triplicate (confirmatory testing). Each plate can assay 28 specimens in the confirmatory testing procedure.
PRINCIPLES OF THE PROCEDURE

The Aware™ BED™ EIA is an IgG-capture enzyme immunoassay (see Figure 1). In this assay, the wells of a microplate are coated with goat anti-human IgG. When serum or plasma is added to the wells, anti-HIV IgG and non-anti-HIV IgG are captured by the goat anti-human IgG. The relative amounts of anti-HIV IgG and non-anti-HIV IgG captured represent IgG antibody populations found in the serum or plasma. Indirectly, the test measures the proportion of HIV-1-specific IgG in a given specimen with respect to total IgG. Early seroconverters have a lower proportion of HIV-specific IgG in their serum/plasma than those with long-term infection [6, 13]. Although the same specimens may have high optical density (OD) values on regular diagnostic EIAs, OD values are lower on the Aware™ BED™ EIA. Studies have indicated that HIV-specific IgG may continue to increase for more than two years after seroconversion when tested by this assay [6].

Figure 1

The specific features of the Aware™ BED™ EIA are:

1. The assay is performed at a 1:101 dilution of the specimen to detect an early or long-term infection. The assay is not affected significantly by a variation in dilution, as long as the proportion of HIV- and non-HIV IgG in the diluted specimen remains constant.
2. The format of the assay results in an ease of dilution and higher precision.
3. The use of a multi-subtype-derived gp41 antigen permits an equivalent detection of antibodies to different HIV-1 subtypes with similar seroconversion durations.
4. The assay can be used in populations with divergent HIV-1 subtypes, using uniform criteria (such as cutoff, seroconversion duration or "window period").
REAGENTS

REFRIGERATOR PACK, PN 98003A (Store Components at 2-8° C)

<table>
<thead>
<tr>
<th>ITEM</th>
<th>COMPONENT - CONTENTS</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>GOAT ANTI-HUMAN IMMUNOGLOBULIN (IgG) COATED MICROWELL PLATE One plate holds twelve (12) 1x8-well strips (96 wells), with adsorbed goat anti-human IgG. Plates are provided in resealable foil pouches with desiccant. (2 Plates - 192 wells)</td>
</tr>
<tr>
<td>2</td>
<td>10X WASH BUFFER CONCENTRATE Contains a 10X concentrate of phosphate buffered saline and detergent. (1 Bottle - 175 mL)</td>
</tr>
<tr>
<td>3</td>
<td>SAMPLE DILUENT Contains a phosphate buffered saline, detergent, bovine serum albumin, and preservative. (1 Bottle - 175 mL)</td>
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<tr>
<td>4</td>
<td>TMB SUBSTRATE 3,3',5,5' Tetramethylbenzidine (TMB) in acidic buffer. (1 Bottle - 27 mL)</td>
</tr>
<tr>
<td>5</td>
<td>STOP SOLUTION 1N H₂SO₄ (1 Bottle - 27 mL)</td>
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<tr>
<td>N/A</td>
<td>PLATE SEALERS Ten plate sealers (1 Package)</td>
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<tr>
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<td>PACKAGE INSERT</td>
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### FREEZER PACK, PN 98003B (Store Components at ≤ -20° C)

<table>
<thead>
<tr>
<th>ITEM</th>
<th>COMPONENT - CONTENTS</th>
</tr>
</thead>
</table>
| **6** | **HIGH POSITIVE CONTROL** (HPC, red cap)  
Inactivated human serum (non-reactive for HBsAg and antibodies to HCV) containing a high titer of antibodies to HIV-1 antigens. Contains preservative. (1 vial - 100 μL) |
| **7** | **LOW POSITIVE CONTROL** (LPC, yellow cap)  
Inactivated human serum (non-reactive for HBsAg and antibodies to HCV) containing a low titer of antibodies to HIV-1 antigens. Contains preservative. (1 vial - 100 μL) |
| **8** | **CALIBRATOR** (CAL, green cap)  
Inactivated human serum (non-reactive for HBsAg and antibodies to HCV) containing a low titer of antibodies to HIV-1 antigens. Contains preservative. (1 vial - 100 μL) |
| **9** | **NEGATIVE CONTROL** (NC, blue cap)  
Inactivated human serum (non-reactive for HBsAg and antibodies to HCV) containing no antibodies to HIV-1 antigens. Contains preservative. (1 vial - 100 μL) |
| **10** | **BIOTINYLATED HIV-1 BED™ PEPTIDE** (BED™, black cap)  
Synthetic peptide structure containing HIV-1 Group B, E and D gp41 epitopes in BSA buffer. (1 vial - 50 μL) |
| **11** | **STREPTAVIDIN-HRP CONJUGATE** (SA-HRP, purple cap)  
Enzyme conjugate in phosphate buffered saline and glycerol. (1 vial - 50 μL) |

### WARNINGS

The Aware™ BED™ EIA is to be used for surveillance purposes only, such as for population incidence estimates to assist in prevention programs, targeting resources, monitoring and evaluation, and identifying high-risk cohorts for prevention research, including vaccine trials. The assay is not for clinical use or for use in the diagnosis of HIV infection.
PRECAUTIONS

1. The use of good laboratory working practices and universal precautions are recommended. Handle assay specimens as if capable of transmitting infectious agents. The serum controls and the calibrator have been inactivated but, as with any human serum, these should be handled as if capable of transmitting infectious agents.

2. Sixty (60) minutes before beginning the assay remove all reagents from refrigerated storage. Bring kit reagents to room temperature (15-30° C) prior to use. Note: remove only as many microwell strips as are needed to perform the test run. Any microwell strips which are not to be used in the current test run should be sealed in the foil bag with desiccant and stored at 2-8° C.

3. Sixty (60) minutes before beginning the assay remove the Calibrator (CAL), the High Positive Control (HPC), the Low Positive Control (LPC), and the Negative Control (NC) from frozen storage. Do not remove the peptide and conjugate from -20°C storage until immediately before use.

4. Return all kit components to their recommended storage conditions immediately after use.

5. Do not interchange bottle or vial caps.

6. Mix all reagents thoroughly just prior to use. All Freezer Pack reagents should be briefly vortexed. All Refrigerator Pack liquid reagents can be mixed by gently inverting 3 to 5 times.

7. Avoid microbial contamination and cross contamination of reagents and specimens. Use separate pipettes and/or pipette tips, and reagent reservoirs for each component of the kit and for each specimen to be tested.

8. Do not use the kit or its reagents beyond the expiration date printed on the kit label.

9. Preparation of 1:101 dilutions of serum controls, calibrator, and specimens requires good mixing. This can be accomplished by extracting, aspirating, and carefully expelling the Sample Diluent, to which the sample has been added, at least 5 times.

10. Replicates of all samples must be independent dilutions.

11. The recommended plate configurations must be used in order to utilize the software spreadsheet (available separately).

12. Wipe spills promptly with a 0.5% sodium hypochlorite solution (1:10 dilution of liquid household bleach). Do not place solutions containing bleach in the autoclave.
STORAGE

The Aware™ BED™ EIA is supplied as two sets of packs.

Refrigerator Pack (PN 98003A) requires refrigeration at 2-8°C. This pack contains the following kit components:

- Goat Anti-Human Immunoglobulin (IgG) Coated Microwell Plate
- 10X Wash Buffer Concentrate
- Sample Diluent
- TMB Substrate
- Stop Solution
- Plate sealers & package insert (stored at room temperature)

Freezer Pack (PN 98003B) requires frozen storage at ≤ -20°C. This pack contains the following kit components:

- High Positive Control (HPC)
- Low Positive Control (LPC)
- Calibrator (CAL)
- Negative Control (NC)
- HIV-1 BED™ Peptide (BED™)
- Streptavidin-HRP Conjugate (SA-HRP)

Sixty (60) minutes before beginning the assay, remove all reagents from refrigerated storage and remove only the Calibrator (CAL), the High Positive Control (HPC), the Low Positive Control (LPC), and the Negative Control (NC) from the freezer. Allow all reagents to reach room temperature (15-30°C) before use. Do not remove the HIV-1 BED Peptide and Streptavidin-HRP Conjugate from the freezer until immediately before use. All reagents should be returned to their labeled storage condition immediately after use.
MATERIALS REQUIRED BUT NOT PROVIDED

- Titertubes for specimen dilution; 1.2 mL tubes arranged 12 x 8 (96 per rack) and compatible with multi-channel pipettes
- Single-channel pipettes and tips capable of delivering 2-20 and 10-100 μL
- Multi-channel pipette and tips capable of delivering up to 200 μL
- Reagent Reservoirs
- 37°C (±2°C) and 25°C (±2°C) incubators
- Vortex Mixer
- Microwell Plate Washer, either 96-well or strip
- Spectrophotometer (Microwell Plate Reader) capable of reading 96-well plates at 450 nm with a reference filter at 630-650 nm
- Stir plate and magnetic stir bar
- Centrifuge tubes, 15 mL polypropylene
- Timer
- Graduated cylinders (100 mL and 1000 mL)
- Deionized water
- Absorbant paper
- Household bleach (5-8% hypochlorite)
- Disposable gloves
- Serological pipettes, pipette bulb or equivalent
- Appropriate-sized beakers and flasks (100 mL, 200 mL, 1 L)

Note: Additional materials are required for testing of dried plasma, serum, and/or blood spots. Consult the Package Insert in the Aware™ BED™ EIA DBS Control Pack (PN 98133) for details.
SPECIMEN COLLECTION AND PREPARATION

1. The Aware™ BED™ EIA can be used with liquid serum or plasma specimens, or with serum, plasma, or blood that has been dried on Schleicher and Schuell/Whatman #903 filter paper (DSS, DPS, DBS) [11].

Note: The test of DPS, DBS, and DSS requires a supplemental pack of controls and the Calibrator in dried format. These controls are not included with the test kit and must be purchased separately from Calypte Biomedical (PN 98133). The controls and the Calibrator required for the testing of liquid specimens is included with the kit.

2. Liquid specimens may be stored at 2-8°C for up to two weeks. For longer intervals, specimens may be frozen (-20°C or lower) prior to testing.

3. Avoid multiple freeze-thaw cycles.

4. Reliability of the test results with grossly lipemic, hemolyzed, or cloudy specimens is not known.

5. Mix specimens well by inversion or vortex. Centrifuge if necessary to remove particulate matter prior to testing.

6. If specimens are shipped, they should be shipped in accordance with requirements for transporting etiological agents.

7. Dried specimens must be maintained in a dry environment.

8. Preparation of liquid and filter paper specimens is described under Assay Procedure below.

REAGENT PREPARATION

1. Remove the main kit from the refrigerator and the kit controls and specimens from the freezer (HIV-1 BED™ Peptide and Streptavidin-HRP Conjugate should remain in the freezer). Allow all reagents to reach room temperature (15-30°C) before use.

2. Prepare 10X Wash Buffer Concentrate as follows:
   a) Mix 100 mL of 10X Wash Buffer Concentrate and 900 mL of deionized water.
   b) Mix well using a magnetic stir bar and stir plate for at least 10 minutes.

Note: The 1X Wash Buffer may be stored at 2-8°C for use within 1 week after which it should be discarded.
ASSAY PROCEDURE
Recommended Plate Configuration – Initial Testing
The recommended plate configuration for initial testing is shown below.
Specimens are tested singly during initial testing.

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</table>

Note: This plate configuration must be used in order to utilize the software spreadsheet available for downloading at the Calypte Biomedical Corporation Internet website at http://www.calypte.com.

Preparation and Addition of Controls, Calibrator, and Test Specimens
The test protocol for liquid or filter paper specimens varies only in the preparation and addition of the controls, Calibrator, and specimens. All other aspects of the test procedure are identical for liquid and filter paper specimens.

Dried Plasma, Dried Blood, or Dried Serum Spots (DPS, DBS, DSS)
Each of the three dried specimen types can be tested simultaneously. There are two methods for the preparation of dried specimens. The method used must be consistent for every control, Calibrator, and specimen on the plate. Choose one method below for the preparation of dried specimens. Both methods require the use of the Aware™ BED™ EIA DBS Control Pack (PN 98133) available from Calypte Biomedical Corp.

Method A: ELISA Plate Elution of DPS, DBS, or DSS
A-1. Obtain an uncoated 96-well ELISA pre-plate.
A-2. Prepare a plate map, selecting 2 microwells to be assigned for the DBS Negative Control and 3 microwells each for the DBS Calibrator, DBS Low Positive Control, DBS High Positive Control Spot, and one well for each of the test specimens. The controls and Calibrator must be run on every plate.
A-3. Using a 6-mm hole punch, punch out two disks from the DBS Negative Control Spot and place each into separate microwells consistent with the plate map.
A-4. Using a 6-mm hole punch, punch out three disks from the DBS Calibrator Spot and place each into separate microwells consistent with the plate map.

A-5. Continue Step A-4 above for the DBS Low Positive Control and DBS High Positive Control.

A-6. Continue by taking punches from specimen cards and placing the disks into separate wells for elution. The DBS controls can simultaneously be used for testing of punched-out disks made from serum, plasma, or whole blood.

A-7. Using a multichannel pipette, add 200 μL of Sample Diluent to each well that contains a DBS Control or specimen disk. As you add the diluent, carefully mix the solution 3 times by expelling and dispensing the diluent in the pipette tips to ensure that discs are submerged. Employ first-stop pipetting to prevent bubbles from forming. Use clean pipette tips for each control or specimen.

A-8. Cover the plate with a plate sealer.

A-9. Incubate the plate at 2-8° C for 12-16 hours.

A-10. Following the elution of the controls and specimens, prepare to run the Aware™ BED™ EIA. Initially, bring all reagents (including the Sample Diluent and the elution plate) to room temperature.

A-11. To test dried specimens eluted in an ELISA plate requires a 1:2 dilution. First, use a multichannel pipette to add 50 μL of Sample Diluent to each well of the test plate.
A-12. Use a multichannel pipette to mix the contents of the eluted materials at least 3 times and then transfer 50 μL of each control, the Calibrator, or test sample from each well of the elution plate to the test plate maintaining the same configuration of samples. Mix the eluted samples with the diluent 3 times in the test plate.

A-13. Proceed with the Aware™ BED™ EIA test protocol (Step 1h, page 14).

Note: Eluted material can be reused up to 2 days if stored at 2 to 8 °C. However, for the confirmation assay, start with new punches and elution procedure (Step A-1).

Method B: Titer Tube Elution of DPS, DBS, or DSS

B-1. Obtain an 8 x 12 titer tube unit and label to define front/back orientation.

B-2. Prepare a plate map, selecting 2 microwells to be assigned for the DBS Negative Control and 3 microwells each for the DBS Calibrator, DBS Low Positive Control, DBS High Positive Control, and one well for each of the test specimens. The controls and Calibrator must be run on every test plate.

B-3. Using a 6-mm hole punch, punch out two disks from the DBS Negative Control Spot and place each into separate titertubes consistent with the plate map. The use of titer tubes for spot elution may require a minor crimping/folding of the disk with a forceps to allow it to completely settle to the bottom of the titertube.

B-4. Using a 6-mm hole punch, punch out three disks from the DBS Calibrator Spot and place each into separate titertubes consistent with the plate map.
B-5. Continue Step B-4 above for the DBS Low Positive Control, and DBS High Positive Control.

B-6. Continue by taking punches from specimen cards and placing the disks into separate titertubes for elution. The DBS controls can be simultaneously used for testing of punched out disks made from serum, plasma, or whole blood.

B-7. Using a multichannel pipette, add 400 μL of Sample Diluent to each tube that contains a DBS Control or specimen disk. As you add the diluent, carefully mix the solution 3 times by expelling and dispensing the diluent in the pipette tips to ensure that discs are submerged. Employ first-stop pipetting to prevent bubbles from forming. Use separate tips for each plate control or specimen.

B-8. Cover the titertube rack with a plate sealer or lid.

B-9. Incubate titertube rack at 2-8° C for 12-16 hours.

B-10. Following the overnight elution of the controls and specimens, prepare to run the Aware™ BED™ EIA. Initially, bring all reagents (including the Sample Diluent and the elution tubes) to room temperature.

B-11. To test dried spot specimens, controls, or the Calibrator eluted in titertubes requires no further dilution. Simply use a multichannel pipette to mix the contents 4-6 times and transfer 100 μL of each control, the Calibrator, or test sample from the elution tubes to the test plate maintaining the same configuration of samples.

B-12. Proceed with the standard Aware™ BED™ EIA test protocol (Step 1h, page 14).

Note: Eluted material can be reused up to 2 days if stored at 2-8 °C. However, for the confirmation assay, start with new punches and elution procedure (Step B-1).
Liquid Plasma and Liquid Serum Specimens

Note: Each of the two liquid types can be tested simultaneously.

1. Prepare 1:101 dilutions of controls and specimens in titertubes using the recommended plate configuration on listed on page 10.
   a) Transfer 500 μL of Sample Diluent to each tube using a multichannel pipette. Add 5 μL control or specimen to designated tube. Return the vial controls and calibrator to the freezer after preparing the required dilutions.
   b) Prepare 2 replicate dilutions of the Negative Control (NC).
   c) Prepare 3 replicate dilutions of the Calibrator (CAL).
   d) Prepare 3 replicate dilutions of the Low Positive Control (LPC).
   e) Prepare 3 replicate dilutions of the High Positive Control (HPC).
   f) Prepare a single dilution of each specimen to be tested.
      Note: For the confirmatory assay, prepare 3 replicate dilutions of each specimen to be confirmed.
   h) Using a multi-channel pipette, carefully mix diluted controls and specimens 4-6 times in the titer tubes and then transfer 100 μL of each diluted control and specimen to test plate (Goat Anti-Human Immunoglobulin (IgG) Coated Microwell Plate).

2. Cover plate with plate sealer. Incubate for 1 hour at 37°C (± 2°C).
   Note: Ten minutes before the end of the sample incubation, prepare the BED™ Peptide working reagent as described in Step 3 below.

3. Make a 1:1001 dilution of HIV-1 BED™ Peptide in Sample Diluent for either a full or partial plate as follows:
   a) For one plate: briefly vortex the vial containing the HIV-1 BED™ Peptide and then add 12 μL of
HIV-1 BED Peptide to 12 mL of the Sample Diluent. Vortex to thoroughly mix.

b) For a partial plate: for each 8-well strip to be used, add 1 μL of HIV-1 BED™ Peptide to 1 mL of the Sample Diluent. Vortex to thoroughly mix.

4. Using a 96-well or strip plate washer, wash plate 4 times (rotating the plate after the first 2 washes) with 300 μL/well of the prepared 1X Wash Buffer, with a 10-second soak between each wash. If strip washer is used, programming it to include soak cycle is not necessary. After the final wash, wrap the plate in absorbant paper and tap upside down to remove any remaining wash buffer.

5. Using a multi-channel pipette, add 100 μL of diluted HIV-1 BED™ Peptide to each well, cover with a plate sealer and incubate 1 hour at 37° C (± 2° C).

6. Dilute Streptavidin-HRP Conjugate (SA-HRP) 1:1001 in the Sample Diluent for either a full or partial plate as follows:

a) For one plate: briefly vortex the vial containing the Streptavidin-HRP Conjugate (SA-HRP) and then add 12 μL to 12 mL of the Sample Diluent. Vortex to thoroughly mix. Return SA-HRP vial to the freezer.

b) For partial plate: for each 8-well strip to be used, add 1 μL of Streptavidin-HRP Conjugate (SA-HRP) to 1 mL of Sample Diluent. Vortex to thoroughly mix. Return SA-HRP vial to the freezer.

7. Wash plate as in step 4.

8. Using a multi-channel pipette, add 100 μL of diluted conjugate to each well, cover with a plate sealer and incubate for 90 minutes at 37° C (± 2° C).

10. Using a multi-channel pipette, add 100 μL of TMB Substrate to each well (do not cover plate).

11. Incubate in a 25°C (±2°C) incubator for exactly 15 minutes for color development.

12. Using a multi-channel pipette, add 100 μL of Stop Solution to each well to stop the reaction.

13. Read plate using a spectrophotometer set at 450 nm wavelength with reference wavelength at 630 to 650 nm.

RUN VALIDATION AND CALCULATION OF RESULTS

Calculate Median Optical Density (OD) Results for Controls and Calibrator
Determine the median OD value for each of the controls and calibrator. The median OD value is the "middle" value (not the average) of the 3 ODs. For example, the median of three values, 0.467, 0.480, and 0.505 is 0.480. The median OD values are used in the calculation of the normalized OD (ODn).

Evaluate the Median OD Values for Controls and Calibrator
The median OD value for each control and calibrator must be within the indicated ranges shown below for a run to be considered acceptable.

Acceptable Median OD Ranges of Controls and Calibrator
If the median OD value of the calibrator or any control falls outside of these limits, the run is invalid and should be rejected and repeated. For the NC, both individual OD values must be within the range for NC acceptance criteria in the initial and confirmatory results.

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<tr>
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<th>CAL</th>
<th>LPC</th>
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</table>
Calculation of Normalized OD Results (ODn)

OD normalization decreases run-to-run variability and increases reproducibility [6, 9]. Determine the normalized OD (ODn) for the median of the observed OD values for each control. The normalized OD (ODn) is calculated by dividing the median OD for each control by the median OD of the Calibrator.

The ODn of control = (median OD of control) divided by (median OD of Calibrator)

The ODn for samples is determined in the same manner.

The ODn of sample #1 = (OD of sample #1) divided by (median OD of Calibrator)

For confirmatory testing, the ODn is the median of the triplicate OD values for the specimen divided by the median OD of the Calibrator.

Minimum/Maximum Acceptable ODn Ranges of Controls and Calibrator

If the ODn of the median of any control or the calibrator falls outside of these limits, the run should be rejected as invalid and repeated.

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<tr>
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<td>Maximum</td>
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<td>1.000</td>
<td>0.750</td>
<td>1.900</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS

All test specimens are initially run singly on the Aware™ BED™ EIA. Specimens with ODn < 1.2 are tested again in triplicate to confirm their ODn values. In confirmatory testing, if the ODn of the specimen is < 0.8, the specimen is considered a recent seroconversion. The algorithm used for testing and interpretation is shown below.

```
if ODn > 1.2
  Long-term seroconversion
else if ODn ≤ 1.2
  Repeat test in triplicate (Confirmatory)
  if ODn > 0.8
    Long-term seroconversion
  else if ODn ≤ 0.8
    Recent seroconversion
```
NOTE: A spreadsheet to facilitate run validation and the calculation of results is available for downloading on the Calypte® Biomedical Corporation internet website at http://www.calypte.com. It is recommended that this spreadsheet be used for all assay runs.

CONFIRMATORY TESTING
When test results are determined to be valid, specimens with ODn < 1.2 require further repeat testing in triplicate using the following recommended plate configuration. For confirmatory testing, follow assay procedure steps 1-13 (page 14-16), preparing three independent replicate dilutions of each specimen to be tested.

Recommended Plate Configuration - Confirmatory Testing
(Note that specimens are tested in triplicate during confirmatory testing.)

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>HPC</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>26</th>
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<tbody>
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<td>8</td>
<td>11</td>
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<td>21</td>
<td>24</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>CAL</td>
<td>HPC</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>11</td>
<td>14</td>
<td>16</td>
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<td>22</td>
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</tr>
<tr>
<td>CAL</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>11</td>
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<td>17</td>
<td>19</td>
<td>22</td>
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<td>27</td>
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<tr>
<td>CAL</td>
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<td>4</td>
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</tr>
<tr>
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<td>4</td>
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<td>21</td>
<td>23</td>
<td>26</td>
<td>BL*</td>
<td></td>
</tr>
</tbody>
</table>

* BL = blank

SPECIFIC PERFORMANCE CHARACTERISTICS
Original findings indicated a cutoff (ODn) of 1.0 represented a mean seroconversion duration of 160 days [6]. Further evaluations and analyses using additional panels with other subtype infections suggest that the ODn of 0.8 corresponds to mean seroconversion duration of 155 days. This cutoff of 0.8 yields few false recent infections in AIDS patients, resulting in a better predictive value for detection of recent infection.

The predictive value of any assay depends on the prevalence of that condition in a population. Therefore, the predictive value of detecting recently infected individuals in low incidence populations would be lower than in higher incidence populations.
Overall performance of the assay has been well studied examining reproducibility, inter-run and intra-run coefficient of variation (CV), and inter-operator variability [9], and data suggest that the assay has very high reproducibility with $R^2 > 0.9$.

The High Positive Control (HPC), Calibrator (CAL), and Low Positive Control (LPC) are prepared such that they have 2-fold differences in their HIV-IgG levels. This information can be used for quality control purposes to generate a Control Plot.

**LIMITATIONS OF THE ASSAY**

Classification of individuals by the Aware™ BED™ EIA as recent seroconverters or long-term infections is based on average timeframes in which individuals develop HIV antibodies calculated from data using a large number of people [6]. However, there are differences among the individuals in the rates at which antibodies are produced. Although this assay is useful at the population level, its predictive value for the individual may be low (especially when ODn levels are close to the cutoff). Therefore, the assay should not be used for individual diagnosis. About 2-3% of people with long-term HIV infections, including AIDS, may be misclassified as recently infected. Efforts should be made to exclude people with AIDS or low CD4 counts to increase the predictive value of the assay.
CALCULATING INCIDENCE
Minor variations of the formula for calculating incidence have been used in earlier studies [1,6]. A consensus formula was agreed upon at the US CDC for calculating incidence. Annual HIV-1 incidence is calculated using the following consensus formula:

\[
I = \frac{(365/w) N_{rc}}{N_{mg} + (365/w) N_{rc}/2} \times 100
\]

\( w = \) window period
\( N_{rc} = \) number recent HIV infection
\( N_{mg} = \) number HIV seronegative

The total number of people tested, number seronegative, number seropositive, and number recently infected must be known for calculating incidence in a given cross-sectional population. Note that calculated incidence does not differ significantly when different formulae are used. However, a consistent approach is recommended for rational comparisons among populations and trend analysis.

The 95% confidence interval (CI) for the Incidence estimate is:

\[
95\% \ CI = I \pm 1.96 \left( \sqrt{\frac{I}{N_{rc}}} \right)
\]

This formula for calculating the 95% CI best fits the observed CI in statistical modeling and is dependent on the calculated incidence and the number found to be recently infected [10].

In 2006, the US CDC released recommendations for use of the BED assay and an adjusted formula (http://www.calypte.com/pdf/adjustment-formula.pdf) that corrects for misclassification for incidence calculation and improves incidence estimation [13, 19]. The recommendations can be downloaded from the following CDC website link: http://www.cdc.gov/hiv/topics/surveillance/resources/factsheets/BED.htm#2

If there is evidence of overestimation of incidence, as observed in some studies, you may seek assistance from CDC as outlined in the recommendations.

For information regarding the use of Aware™ BED™ EIA to estimate incidence using STARHS in the context of surveillance in the United States, please visit CDC website: http://www.cdc.gov/hiv/topics/surveillance/resources/factsheets/pdf/bed.pdf
EXPLANATION OF SYMBOLS USED

The following symbols may appear in the labeling (insert and/or labels) for Calypte® products and are derived from the following international standards and other documents:

ANSI/AAMI/ISO 15223, “Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied”.

BS EN 980, “Graphical symbols for use in the labeling of medical devices”.

“Guidance for Industry and FDA Staff - Use of Symbols on Labels and in Labeling of In Vitro Diagnostic Devices Intended for Professional Use”.

- Consult instructions for use
- Catalog number (part number)
- Batch code (lot number)
- Manufacturer
- Temperature limitation (storage temperature)
- Upper limit of temperature (maximum storage temperature)
- Corrosive (acid)
- Use by (expiration date)

Other symbols and abbreviations used:

PN: Part Number  NA: Not Applicable  LN: Label Number
BIBLIOGRAPHY


12. Parekh BS. Use of dried blood spot (DBS) or dried serum/plasma spot (DSS/DPS) specimens to detect recent HIV-1 seroconversion by the BED-CEIA. Oral presentation, CDC/APHL Conference on HIV Diagnostics: New Developments and Challenges, Orlando, February 28 – March 1, 2005.
http://www.hivtestingconference.org/hivtesting2005/Session5/Parekh.pps


