Sedia™ BED HIV-1 Incidence EIA

Enzyme Immunoassay for Population Estimates of HIV-1 Incidence

Cat. No. 1000

FOR RESEARCH USE ONLY
not intended for use in diagnostic procedures

Includes Two Packs:
3012 - Refrigerator Pack (Store at 2 to 8°C)
3013 - Freezer Pack (Store at -25°C to -10°C)

Manufactured in the USA
Sedia Biosciences Corporation
Portland, Oregon USA
+1-(503)-459-4159

Copyright 2014. Sedia Biosciences Corporation
All rights reserved
NAME AND INTENDED USE:
The Sedia™ BED HIV-1 Incidence EIA is an in vitro quantitative enzyme immunoassay for distinguishing recent HIV-1 infections from those which are long-term. The test measures the proportion of HIV-1 specific IgG to total IgG in blood samples including plasma, serum, and dried blood, plasma, and serum spots [1, 2]. The Sedia™ BED HIV-1 Incidence EIA is solely intended for research use only such as estimating HIV-1 incidence in a population, monitoring and evaluating intervention programs, and recognizing those high-incidence populations so that prevention research, vaccine trials, and resources are most appropriately utilized. This product is not intended for use in diagnostic procedures or for determining clinical outcome or treatment.

BACKGROUND OF HIV-1 INCIDENCE TESTING
The value of HIV-1 incidence testing as an epidemiological tool for intervention programs, resource management, and detailed population surveillance was recognized relatively recently. The early methods first published in 1998 [3] employed the use of desensitized commercial HIV immunoassays. The lower titers of anti-HIV antibodies in recently infected individuals provided the scientific rationale for the use of less sensitive tests as a means of investigating HIV incidence. Various methods based on this theme and alternative scientific approaches such as the differential avidity, affinity, and specificity of the HIV-1 antibody in recent and long-term infections have been investigated [4]. Of the methods evaluated, the less sensitive EIA approach was most pursued and used by some U.S. laboratories to measure HIV-1 incidence [5]. However, because less sensitive EIAs are developed by modifying the procedure of commercial HIV-1 EIAs that employ HIV-1 subtype B antigen(s), these tests were found to be more accurate in populations with primarily subtype B infections [6, 7]. This subtype bias liability, as well as the 1:20,000 sample dilution, assay variability, and other disadvantages associated with the use of less sensitive EIAs for incidence testing spurred development of improved methods.

Scientists at the United States Centers for Disease Control and Prevention (US CDC) developed a second-generation HIV-1 Incidence assay to differentiate recent from long-term HIV-1 infections [1]. The CDC test, utilizing a custom “BED” peptide antigen that includes divergent gp41 sequences from all HIV-1 (group M) subtypes and recombinants, minimizes many of the weaknesses and limitations associated with HIV-1 incidence testing using the less sensitive EIAs [1]. Since it’s inception, the BED EIA has been used in several cross-sectional populations to estimate incidence and evaluate association with various risk factors [8, 9,10, 11, 12, 13, 14, 15, 16, 17]. In 2004, the CDC began to license the BED EIA technology for commercial manufacture and distribution. In 2006, the BED EIA was adopted for use in a national surveillance program in the United States [18]. Sedia Biosciences distributes the Sedia™ BED HIV-1 Incidence EIA under license to and in conformance with the specifications of the CDC.
SUMMARY AND EXPLANATION OF THE TEST
The Sedia™ BED HIV-1 Incidence EIA measures the ratio of HIV-1 specific immunoglobulin G (IgG) to total IgG in a serum or plasma specimen and determines the recent/long term HIV-1 status by referencing the EIA numerical result against that of an internal calibrator specimen. The principle of the test is based on the observation that the ratio of HIV-specific IgG to total IgG is lower in early HIV-1 seroconverters than those with a longer term infection [1, 20]. In fact, studies have suggested that HIV-specific IgG increases for more than two years after seroconversion when tested by the BED EIA [1].

PRINCIPLES OF THE PROCEDURE
1. The Sedia™ BED HIV-1 Incidence EIA is an IgG-capture enzyme immunoassay (C-EIA). During a sample incubation of 60 min. at 37°C, human IgG (including HIV-specific IgG) is captured by goat anti-human IgG coated to the wells of a microplate.

2. Biotinylated HIV-1 BED peptide is incubated for 60 min at 37°C. The peptide is captured only by anti-HIV IgG.

3. Streptavidin-labelled HRP is incubated for 90 min at 37°C and binds to the biotin.

4. TMB substrate is incubated at 25°C and color is generated with intensity proportional to the amount of HRP.

5. The optical density of each well is measured. The OD value, consistent with the proportion of HIV-specific IgG to total IgG, is divided by the OD value of an internal kit calibrator to generate the normalized OD or “ODn”. The value of the ODn dictates whether a result needs to be confirmed and/or if the HIV infection is recent or long term.
FEATURES OF THE SEDIA™ BED HIV-1 INCIDENCE EIA

- The assay principle is based on the more relevant ratio of HIV-1 specific IgG to total IgG.
- The assay does not rely on dilution schemes of 1:20,000 or more which increases the accuracy and reproducibility of the assay.
- The simple 1:101 sample dilution step can be accomplished in a single step.
- The assay uses microtiter plates with removable 1x8 strips and individual wells, allowing the use of exactly the number of wells needed, thus eliminating waste.
- The assay is compatible with liquid plasma and serum as well as dried specimens from serum, plasma, and blood. (Supplemental controls kit from Sedia is required for testing of dried specimens, Cat. No. 1001).
- Serum controls and Calibrator have been heat inactivated.
- The assay is manufactured to the exact specifications of the United States CDC by scientists with significant experience in HIV-1 incidence test development and manufacture.
- Each lot of the Sedia™ BED HIV-1 Incidence EIA has gone through a stringent QC check before release to customers.

GENERAL KIT INFORMATION

The Sedia™ BED HIV-1 Incidence EIA is comprised of two components of matching lot numbers that have separate temperature requirements (frozen and refrigerated). The kit contains two 96-well plates with twelve (12) 1 x 8 removable strips and all necessary reagents to run the assay. Each 1 x 8 strip may be further broken down into individual wells which may be inserted back into the plate frame so that only the exact number of wells required need be used. It is recommended that empty spaces in the plate frame be filled with used wells or strips if using dispensing and/or washing equipment that cannot be programmed on an individual well basis.

Each test plate requires 11 wells be allocated for the controls and Calibrator thus allowing for the test of up to 85 specimens in the initial screening mode. Specimens generating an ODn value below a threshold value must be confirmed by triplicate testing in a subsequent test run following the same procedure. Twenty eight (28) specimens can be tested on each plate in the confirmatory testing mode.
MATERIALS PROVIDED AND THEIR STORAGE

REFRIGERATOR PACK (#3012): Store contents at 2 to 8°C.

1. Goat Anti-Human Immunoglobulin (IgG)-Coated Microwell Plates - Two (2) 96-well plates. Plates are provided in resealable foil pouches with desiccant.

2. 10X Wash Buffer Concentrate - Two (2) bottles (100 mL). Contains phosphate buffered saline, detergent and preservative. Note: BED EIA 10X Wash Buffer Concentrate is identical to Sedia’s HIV-1 LAg-Avidity EIA (Cat. No. 1002) 10X Wash Buffer Concentrate, and may be used interchangeably regardless of lot number if both assays are being run by the lab.

3. Sample Diluent - One (1) bottle (175 mL). Contains phosphate buffered saline, detergent, blocking agents and preservative.

4. TMB Substrate - One (1) bottle (27 mL). Contains 3,3’,5,5’ tetramethylbenzidine (TMB) in acidic buffer.

5. Stop Solution - One (1) bottle (27 mL). Contains dilute acid solution.

6. Plate Sealers - One (1) pack of 25.

7. Package Insert - One (1).

FREEZER PACK (#3013): Store contents at -25°C to -10°C.

6. Negative Control (NC, blue cap) - One (1) vial (100 µl). Inactivated human serum non-reactive for HBsAg and antibodies to HCV and HIV. Contains preservative.

7. Calibrator (CAL, green cap) - One (1) vial (100 µl). Inactivated human serum reactive to HIV-1 antigens. Non-reactive for HBsAg and antibodies to HCV. Contains preservative.

8. Low Positive Control (LPC, yellow cap) - One (1) vial (100 µl). Inactivated human serum reactive to HIV-1 antigens. Non-reactive for HBsAg and antibodies to HCV. Contains preservative.

9. High Positive Control (HPC, red cap) - One (1) vial (100 µl). Inactivated human serum reactive to HIV-1 antigens. Non-reactive for HBsAg and antibodies to HCV. Contains preservative.

10. HIV-1 BED PEPTIDE (BED, black cap) - One (1) vial (50 µl). Biotinylated synthetic peptide structure that includes divergent gp41 sequences from all HIV-1 (group M) subtypes and recombinants. In BSA buffer with preservative. Concentrated to 1001X.

11. Streptavidin-HRP conjugate (SA-HRP, purple cap) - One (1) vial (50 µl). Streptavidin conjugated to horseradish peroxidase (HRP). Contains preservative and is concentrated to 1001X the Conjugate Working Solution.
WARNINGS AND PRECAUTIONS

1. The Sedia™ BED HIV-1 Incidence EIA is intended for research use only, specifically for the determination of the incidence of HIV-1 infection in certain populations. It is not intended for use in diagnostic procedures or for determining clinical outcome or treatment.

2. The use of universal precautions and good laboratory working practices are strongly recommended. Although the kit’s serum controls and Calibrator have been inactivated, these reagents and any clinical specimens should be handled as if capable of transmitting infectious agents.

3. It is critical that all aspects of the procedure be strictly adhered to, particularly timing and temperatures. Test components must not be used after their expiration date.

4. The Sedia™ BED HIV-1 Incidence EIA is comprised of two packs, a Freezer Pack (3013) and a Refrigerator Pack (3012)) with matching lot numbers. It is critical that only packs with matching lot numbers be used together during the operation of the test. The 10X Wash Buffer is lot number independent and may be used with other BED kit lots and with the Sedia™ HIV-1 LAg-Avidity EIA if both assays are being run at the same time.

5. For accurate results, dried blood, plasma and serum test specimens must be collected only on FDA-approved filter papers, i.e. Whatman #903 or Ahlstrom Grade 226.

6. If the Sedia™ BED HIV-1 Incidence EIA is used to test dried blood, plasma or serum test specimens, only Sedia™ Dried Blood Spot Controls (Cat. #1001) should be used to perform the assay.

7. Promptly disinfect any spills using a 0.5% sodium hypochlorite solution (1:10 dilution of liquid household bleach) or equivalent. Thoroughly clean work surface both before and after the procedure.

8. Use plate washers, readers and incubators that have undergone proper installation, operational and performance qualification and are properly maintained to minimize assay variability. It is important to read results on a plate reader at 450 nm against a reference filter at 620-650 nm to obtain accurate OD and ODn values within acceptable ranges.

IMPORTANT PROCEDURAL NOTES

1. Do not use kit or kit components beyond the expiration dates specified on the product and component labeling. Storage of kit materials at temperatures except as specified may result in diminished assay performance and may give inaccurate results.

2. Remove the Refrigerator Pack and the kit controls and Calibrator in the Freezer Pack from cold storage 60 minutes before the test is to be run. Allow all reagents to reach room temperature (15-30° C) before use. The HIV-1 BED Peptide and Streptavidin-HRP reagents should remain in the freezer until immediately before use. Place the TMB Substrate bottle in a 25° C incubator until use.

3. If using less than 96 wells, use only the required number of strips and/or wells and return unused strips/wells to the foil bag with desiccant, seal, and store at 2-8° C.
4. Use only calibrated pipettes. Always use separate pipette tips, tubes and reagent reservoirs for each specimen or kit component. Do not interchange bottle or vial caps.

5. Mix all reagents immediately before use. Refrigerator Pack reagents can be mixed by gentle inversion 3-5 times. Freezer Pack reagents should be briefly vortexed. Wash Buffer Concentrate typically requires additional both mixing and warming in a water to dissolve salt crystals. The Streptavidin-HRP conjugate concentrate may need to be flicked or spun down after vortexing as it often congregates in the cap.

6. Preparation of 1:101 dilutions of specimens, serum controls and the kit Calibrator requires thorough mixing and accurate measurement. Carefully mix the Sample Diluent with the sample in the titer tube by drawing the mixture up and down in the multichannel pipette at least 4 times. Pipette slowly to avoid bubbles. Change pipette tips for each specimen. Filtered tips are recommended for specimen handling to minimize cross-contamination. Unfiltered tips may be used for all subsequent steps.

7. The kit and its components should be returned to their recommended storage conditions after use.

MATERIALS REQUIRED BUT NOT PROVIDED

- Polypropylene tubes with cap, 12-15 mL.
- Graduated cylinders, beakers and flasks.
- Serological pipettes with pipette bulb (or equivalent).
- Single-channel pipettes (2-100 µL) and multi-channel pipette (100-200 µL). Filtered tips recommended for specimen handling to minimize cross-contamination. Positive displacement pipettes or microliter syringes are recommended for measuring out the SA-HRP conjugate concentrate.
- Reagent reservoir (e.g. VWR International, 800-932-5000, www.vwr.com, catalog number 53504-035 or equivalent).
- Incubators, one each at 37° C (±2° C) and 25°C (±2° C).
- Vortex Mixer.
- Microwell Plate Washer, either 96-well or strip.
- Spectrophotometer (Plate Reader) capable of reading 96-well plates at 450 nm with a reference filter at 620-650 nm.
- Timer.
- Deionized water.
- Household bleach (5-8% hypochlorite).
- Personal protection equipment (PPE) (disposable gloves, safety glasses, etc.).
- Biohazardous waste container.

Note: Testing of dried plasma, serum, and/or blood spots requires additional materials included in the Sedia™ BED EIA DBS Controls Pack (catalog number 1001). Refer to the DBS Controls Package Insert for details.
SPECIMEN COLLECTION AND PREPARATION

1. The Sedia™ BED HIV-1 Incidence EIA is intended for use with liquid serum or plasma specimens, or with serum, plasma, or blood that has been dried on Ahlstrom Grade 226 paper cards or GE (Whatman) #903 filter paper (DSS, DPS, and DSS). [2, 19].
   Note: The test of DBS, DPS, and DSS specimens requires a supplemental pack of Sedia™ BED HIV-1 Incidence EIA controls and Calibrator in dried format. These controls must be purchased separately from Sedia Biosciences (Sedia™ BED HIV-1 Incidence Dried Blood Spot Controls Kit, Catalog No. 1001).

2. Liquid specimens may be stored at 2-8° C for up to two weeks. Longer-term storage should be at frozen conditions (-20° C or lower).

3. Specimens diluted in Sample Diluent should be tested within 24 hours of dilution. If retesting is required, fresh dilutions should be prepared prior to testing.

4. Multiple freeze-thaw cycles should be avoided for specimens.

5. Test performance with grossly hemolyzed, lipemic or cloudy specimens has not been determined.

6. Specimens should be mixed well by inversion or vortex before testing. Particulate matter can be removed by appropriate centrifugation.

7. Specimens should be shipped in accordance with all local requirements and regulations for the transport of etiological agents.

8. Dried specimens must be maintained in a dry environment. Storage in a sealed pouch with desiccant and humidity indicator card is recommended.

9. The test of dried specimens requires an alternative procedure for sample preparation (described under Assay Procedure below and in the product Insert of the Sedia™ BED HIV-1 Incidence Dried Blood Spot Controls Kit, Catalog #1001).

REAGENT PREPARATION

1. Remove the Refrigerator Pack and the kit controls and Calibrator in the Freezer Pack from cold storage 60 minutes before the test is to be run. Allow all reagents to reach room temperature (15-30° C) before use except as per note 2 below.

2. The HIV-1 BED Peptide and Streptavidin-HRP reagents should remain in the freezer until immediately before use. Place the TMB Substrate bottle in a 25° C incubator until use. Return all kit components to their recommended storage conditions immediately after use.

3. Prepare 1X Wash Buffer 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.

   Notes: The 10X Wash Buffer Concentrate may require brief heating in a water bath to dissolve remaining salt crystals.
   10X Wash Buffer Concentrate from Sedia HIV-1 LAg-Avidity EIA (Cat. No. 1002) may also be used in this assay. See page 5 above for details.
   Store the 1X Wash Buffer at 2-30° C for a maximum of 1 month and then discard.

4. The HIV-1 BED Peptide and Streptavidin-HRP Conjugate may need to be flicked or spun down after vortexing as they often congregate in the cap after vortexing.
ASSAY PROCEDURE

1.0 Creation of Plate Diagram or Map

The plate configuration shown below is recommended for the initial testing. Note that the Negative Control is tested in duplicate, the two other controls and Calibrator are tested in triplicate, and (during the initial testing) all specimens are tested singly. For confirmatory testing, the controls are tested in the same manner but each specimen is tested in triplicate (see plate map on page 20).

<table>
<thead>
<tr>
<th></th>
<th>HPC</th>
<th>6</th>
<th>14</th>
<th>22</th>
<th>30</th>
<th>38</th>
<th>46</th>
<th>54</th>
<th>62</th>
<th>70</th>
<th>78</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td></td>
<td>7</td>
<td>15</td>
<td>23</td>
<td>31</td>
<td>39</td>
<td>47</td>
<td>55</td>
<td>63</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>NC</td>
<td></td>
<td>8</td>
<td>16</td>
<td>24</td>
<td>32</td>
<td>40</td>
<td>48</td>
<td>56</td>
<td>64</td>
<td>72</td>
<td>80</td>
</tr>
<tr>
<td>CAL</td>
<td></td>
<td>1</td>
<td>9</td>
<td>17</td>
<td>25</td>
<td>33</td>
<td>41</td>
<td>49</td>
<td>57</td>
<td>65</td>
<td>73</td>
</tr>
<tr>
<td>CAL</td>
<td></td>
<td>2</td>
<td>10</td>
<td>18</td>
<td>26</td>
<td>34</td>
<td>42</td>
<td>50</td>
<td>58</td>
<td>66</td>
<td>74</td>
</tr>
<tr>
<td>LPC</td>
<td></td>
<td>3</td>
<td>11</td>
<td>19</td>
<td>27</td>
<td>35</td>
<td>43</td>
<td>51</td>
<td>59</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>LPC</td>
<td></td>
<td>4</td>
<td>12</td>
<td>20</td>
<td>28</td>
<td>36</td>
<td>44</td>
<td>52</td>
<td>60</td>
<td>68</td>
<td>76</td>
</tr>
<tr>
<td>LPC</td>
<td></td>
<td>5</td>
<td>13</td>
<td>21</td>
<td>29</td>
<td>37</td>
<td>45</td>
<td>53</td>
<td>61</td>
<td>69</td>
<td>77</td>
</tr>
</tbody>
</table>

Note: The recommended plate configuration is compatible with the Excel spreadsheet available for downloading at http://www.sediabio.com/products/bed-eia.

Preparation of Controls, Calibrator and Test Specimens: Overview

The Sedia™ BED HIV-1 Incidence EIA can test serum and plasma liquid specimens (simultaneously) as well as dried (filter paper) specimen spots prepared from serum, plasma and blood (DSS, DPS, DBS), also simultaneously. However, liquid and dried specimens can not be tested on the same plate because each specimen type requires kit controls and the Calibrator to be in the same form as the specimen (liquid or dried). The Sedia™ BED EIA contains controls and the Calibrator in liquid form. Controls and the Calibrator in dried form are available for purchase from Sedia Biosciences (Cat. #1001). All aspects of the EIA procedure and data interpretation are identical for liquid and dried specimens except for elution and preparation and the initial set up and dilution of the specimens, controls and Calibrator.

2.0 Preparation and Addition of Dried Filter Paper Controls and Specimens

(Skip this section and proceed to Section 3.0 if liquid specimens are tested)

Elution and preparation of dried specimens and controls can be done using one of two methods: (1) untreated ELISA pre-plate or (2) titertubes (8 x 12 configuration). The methods vary only by the volume of Sample Diluent used during the process and dilution of eluted controls and specimens (described below). The method used must be consistent for every control, Calibrator, and dried test specimen to be tested on a single plate. Before starting, decide whether to elute the dried specimens in either the wells of an untreated ELISA pre-plate or titertubes.
2.1 The plate map or diagram created on the previous page for the Sedia™ BED HIV-1 Incidence EIA plate will also represent the scheme for the elution of the dried controls, specimens and Calibrator. Setup an appropriately labeled blank uncoated 96-well microtiter plate as a “pre-plate” for elution. Alternatively, use titertubes in an 8 x 12 rack.

2.2 Remove a foil pack containing the DBS Controls Cards from the freezer and allow it to come to room temperature before opening. When open, verify that the included humidity indicator card is still blue confirming that the integrity of the cards has not been compromised due to moisture. If the humidity indicator card is pink, do not use the DBS control cards in the package and instead open a fresh foil pouch of controls.

2.3 Use a 6 mm hole punch for all sample, control and Calibrator disks. Punch out two disks from the blue DBS Negative Control Spot on the DBS Controls Card and place each disk into separate microwells of the pre-plate or titertubes consistent with the plate map generated in Step 1.0.

2.4 Punch out three discs from the DBS Calibrator Spot on the green DBS Control Card and place each disk into separate microwells of the pre-plate or titertubes consistent with the map.
2.5 Punch out three disks from the yellow DBS Low Positive Control Spot on the DBS Controls Card and place each disk into separate microwells of the pre-plate or titertubes consistent with the map.

![Low Positive (YELLOW)](image)

2.6 Punch out three discs from the red DBS High Positive Spot on the DBS Control Card and place each disk into separate microwells of the pre-plate or titertubes consistent with the map.

![High Positive (RED)](image)

2.7 Punch a single spot out for each dried specimen and add to a separate microwell or titertube of the pre-plate consistent with the map.

![Specimens](image)

2.8 Once all disks from all specimens and controls have been placed into the untreated ELISA pre-plate or the titertubes, add Sample Diluent following the procedure for either the ELISA Pre-Plate or Titertube Elution method. Ensure all disks are fully submerged in Sample Diluent.

**ELISA PRE-PLATE ELUTION:** Use a multi-channel pipette to add 200 µL of Sample Diluent to each well of the ELISA pre-plate that contains a DBS control or specimen disk.

![Sample Diluent](image)
**TITERTUBE ELUTION:** Use a multichannel pipette to add **400 µL** of Sample Diluent to each titertube that contains a DBS control or specimen disk.

2.9 Cover the ELISA pre-plate or titertube rack. Incubate at 2 to 8°C for 12 to 16 hours to allow for elution.

2.10 Following the elution incubation, prepare to run the Sedia™ BED HIV-1 Incidence EIA as described in the EIA Product Insert. Initially, bring all reagents and the elution pre-plate or titertubes to room temperature.

*Addition of eluted specimens and controls to the Sedia™ BED HIV-1 Incidence EIA plate is different for the titertube and ELISA pre-plate elution methods. Perform one of the following sets of instructions (2.11A or 2.11B).*

2.11A Addition of eluted controls and specimens from an ELISA pre-plate:

1. **Testing dried specimens eluted in an ELISA pre-plate requires a 1:2 dilution.** Use a multichannel pipette to add 50 µL of Sample Diluent to each well of the Sedia™ BED HIV-1 Incidence EIA plate.

2. Use a multichannel pipette to mix the eluted Controls and specimens by carefully pipetting up and down 4 times in the pre-plate. Pipette slowly to prevent bubbles. Use of filtered tips is recommended.

3. Transfer 50 µL from each well of the elution pre-plate to the corresponding well in the BED EIA test plate maintaining the same configuration as the map. Mix the eluted samples with the Sample Diluent in the test plate by carefully drawing the liquid in the assay plate wells up and down 3 times. Pipette slowly to prevent bubbles. Proceed to Step 2.12 on page 13.
2.11B Addition of eluted controls and specimens from titertubes:

1. **Testing dried specimens eluted in titertubes does not require any further dilution.** Use a multichannel pipette to mix the eluted Controls and specimens by carefully pipetting up and down 4 times in the titertubes. Pipette slowly to prevent bubbles.

2. Transfer 100 µL from each titertube to the corresponding well in the test plate maintaining the same configuration of samples consistent with the plate diagram or map.

2.12 Following the addition of eluted controls and specimens from either the ELISA pre-plate or the titertubes to the BED EIA test plate, the plate is ready for incubation. Proceed with the Sedia™ BED HIV-1 Incidence EIA test protocol for the incubation of sample and controls (Test Procedure 4.0, page 14).

*Note: Eluted material can be reused up to 48 hours if stored at 2 to 8°C. However, for the confirmation assay, repeat the elution procedure with new punches and elution incubation (Step 1.0).*

### 3.0 Preparation and Addition of Liquid Serum or Plasma Specimens

Liquid serum or plasma specimens must be diluted 1:101 in 1.2 mL polypropylene micro-dilution tubes (“titertubes”) in an 8x12 rack consistent with the recommended plate map on page 9. Perform this dilution as follows:

3.1 Use a multichannel pipette to transfer 500 µL Sample Diluent to each titertube in the 8x12 rack.

3.2 Use a pipette to transfer 5 µL of each control, Calibrator, and sample to separate titertubes containing 500 µL Sample Diluent. Use a new pipette tip for each control, calibrator or sample to avoid cross-contamination. Sample addition must be consistent with the plate map. Return controls and Calibrator to -20° C storage after use. Liquid plasma and liquid serum specimens can be tested simultaneously.
3.2.1 Prepare 2 separate replicate dilutions of the Negative Control (NC).

3.2.2 Prepare 3 separate replicate dilutions of the Calibrator (CAL).

3.2.3 Prepare 3 separate replicate dilutions of the Low Positive Control (LPC).

3.2.4 Prepare 3 separate replicate dilutions of the High Positive Control (HPC).

3.2.5 Prepare a single dilution of each specimen to be tested.

Note: Confirmatory testing requires specimens be tested in triplicate. See Section 7, page 19.

3.3 Carefully mix the controls and specimens in the titertubes by gently withdrawing and expelling the fluid in the tips of a multichannel pipette 4-6 times. Avoid bubbles by proceeding slowly. Change pipette tips for each sample.

3.4 After mixing, use a multichannel pipette to transfer 100 µL of each specimen or control from the titertubes to the BED test plate. Be sure to use a new pipet tip for each specimen or control. If the frame for the BED plate contains positions that do not contain controls or specimens, it is recommended to fill those positions used “blank” wells or strips.

4.0 Test Procedure

4.1 Apply a plate sealer to the plate and incubate for 1 hour at 37° C (± 2° C).

Note: Proceed to step 4.2 ten (10) minutes before the end of the sample incubation.
4.2 Retrieve the HIV-1 BED Peptide concentrate (BED) from -20° C storage.

4.3 Briefly vortex the vial and transfer 12 µL of the BED Peptide concentrate to a tube containing 12 mL Sample Diluent to prepare the 1:100 dilution. Flick or spin down the BED Peptide tube to make sure the volume is at the bottom before transferring the 12 µL. (BED Peptide is viscous. Pipette slowly to ensure that the proper volume is transferred).

Note: For a partial plate, prepare 1 mL for each strip to be used plus an extra mL (e.g. pipette 7 µL into 7 mL for 6 strips). In any event, a minimum of 6 mL should be prepared to ensure accurate volume measurement.

4.4 Cap and vortex the tube.

4.5 Transfer the diluted BED Peptide to a reagent reservoir.

4.6 After the 1 hour sample incubation, wash the plate 4 times (rotating the plate after the first 2 washes) with 1X Wash Buffer (see Reagent Preparation, page 8) using a 96-well or strip plate washer. Set the washer to dispense 300 µL per well with a 10-second soak. Soaking is not required if a strip washer is used. After the wash is complete, remove any residual buffer in the wells by wrapping the plate in absorbent paper and gently tapping it upside down.

4.7 Transfer 100 µL per well of the diluted BED Peptide from the reservoir to the washed BED EIA test plate using a multichannel pipette.

4.8 Apply a plate sealer to the plate with diluted BED Peptide and incubate for 1 hour at 37° C (± 2° C).

Note: Proceed to step 4.9 ten (10) minutes before the end of the BED Peptide incubation.
4.9 Retrieve the Streptavidin-HRP conjugate concentrate (SA-HRP) from -20° C storage.

4.10 To prepare the Conjugate Working Solution, briefly vortex the vial and transfer 12 µL of the SA-HRP concentrate to a tube containing 12 mL Sample Diluent to prepare the 1:1001 dilution. Flick or spin down the SA-HRP tube to make sure the volume is at the bottom before transferring the 12 µL. (SA-HRP is viscous. Pipette slowly to ensure that the proper volume is transferred. Use of a positive displacement pipette or microliter syringe is recommended.)

Note: For a partial plate, prepare 1 mL for each strip to be used plus an extra mL (e.g. transfer 7 µL into 7 mL for 6 strips). In any event, a minimum of 6 mL should be prepared to ensure accurate volume measurement.

4.11 Cap the tube and vortex or mix by gently inverting the tube 6-8 times.

4.12 Transfer the Conjugate Working Solution to a reagent reservoir.

4.13 After the 1 hour BED Peptide incubation, wash the plate 4 times with 1X Wash Buffer as described in Section 4.6, page 15.

4.14 Transfer 100 µL per well of the Conjugate Working Solution from the reservoir to the washed BED test plate using a multichannel pipette.

4.15 Apply a plate sealer to the plate with Conjugate Working Solution and incubate for 1.5 hour at 37° C (± 2° C).
4.16 After the 1.5 hour Conjugate Working Solution incubation, wash the plate 4 times with 1X Wash Buffer as described in Section 4.6, page 15.

4.17 Take the TMB Substrate out of the 25° C incubator and transfer to a reagent reservoir. Use a multichannel pipette to transfer 100 µL of TMB Substrate to each well.

4.18 Incubate the plate with TMB Substrate for **EXACTLY** 15 minutes at 25° C (± 2° C). Do not cover the plate with a plate sealer.

4.19 Following the 15 minute TMB Substrate incubation, stop the reaction by adding 100uL of Stop Solution per well using a multichannel pipette.

*Note: Do not wash the plate before adding the Stop Solution.*

4.20 Immediately after the addition of Stop Solution, use a spectrophotometer to read the plate at a wavelength of 450 nm **using a reference filter wavelength of 620-650 nm.**
5.0 Run Validation and Calculation of Results

5.1 Calculate the median (not mean) optical density (OD) for each Control and the Calibrator. The median value of three OD values will be the “middle” value. For example, 0.790 would be the median of these three OD values: 0.790, 0.834, and 0.775. The median value of the two Negative Control OD values will be their average.

5.2 Determine if the median OD values fall within the acceptable range specified for each Control and the Calibrator.

### Acceptable OD Ranges

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>CAL</th>
<th>LPC</th>
<th>HPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimum</strong></td>
<td>0.000</td>
<td>0.380</td>
<td>0.200</td>
<td>0.600</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>0.250</td>
<td>1.350</td>
<td>0.800</td>
<td>2.100</td>
</tr>
</tbody>
</table>

If the median OD value of any of the Controls or the Calibrator is not within the ranges above, the run is invalid and must be repeated using fresh specimen dilutions. *Note: both individual OD values for the Negative Control must be within the stated range.*

5.3 Determine the normalized OD results (ODn) for each Control, the Calibrator, and each specimen. The ODn is calculated by dividing the OD value by the median OD of the Calibrator. Studies performed by the US CDC have demonstrated OD normalization by an internal calibrator decreases run-to-run variability and increases reproducibility [1, 20].

\[
ODn = \frac{(OD \text{ of sample, Control, or Calibrator})}{(median \text{ OD of Calibrator})}
\]

*Note: For confirmatory testing, the “OD of the sample” is the median value of the three replicate-tested specimen OD values.*

5.4 Determine if the ODn value for each Control and the Calibrator falls within the acceptable range listed below. If the ODn of any Control falls outside of its range, the run is invalid and must be repeated using fresh specimen dilutions.

### Acceptable ODn Ranges

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>CAL</th>
<th>LPC</th>
<th>HPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minimum</strong></td>
<td>0.000</td>
<td>1.000</td>
<td>0.400</td>
<td>1.200</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>0.300</td>
<td>1.000</td>
<td>0.750</td>
<td>1.900</td>
</tr>
</tbody>
</table>

5.5 A customized spreadsheet is available from the CDC and at [http://www.sediabio.com/products/bed-eia](http://www.sediabio.com/products/bed-eia) to validate the run and calculate ODn values.
6.0 Interpretation of Assay Results

6.1 During the screening mode, the ODn value for each specimen (calculated in step 5.3) dictates whether the specimen is considered a “long-term” infection or whether it must be subjected to confirmatory testing. Specifically, if the ODn of a specimen is >1.2 during the screening mode, no further testing is required and the specimen is considered a long-term infection. If the ODn of a specimen is ≤ 1.2 during the screening mode, then the specimen must be subjected to confirmatory testing (Section 7.0).

During confirmatory mode, if the ODn of a specimen is ≤ 0.8, then the specimen is considered a recent seroconversion. If the ODn is > 0.8 then the specimen is considered a long-term seroconversion. Below is the algorithm used for testing and interpretation.

7.0 Confirmatory Testing

7.1 As presented in the previous section, if the ODn of a specimen is ≤ 1.2 during the screening mode, then the specimen must be subjected to confirmatory testing. Confirmatory testing entails repeating the Sedia™ BED HIV-1 Incidence EIA protocol exactly as specified in Sections 1.0 through 5.0 except that specimens are tested in triplicate rather than singly. The recommended plate configuration is presented on the following page. Note that 28 specimens can be tested in the Confirmatory mode and that one well is intentionally left blank.
### Confirmatory Testing: Recommended Plate Configuration

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Number of subjects</th>
<th>ω (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B (U.S.)</td>
<td>273</td>
<td>162 (146, 179)</td>
</tr>
<tr>
<td>C (Africa)</td>
<td>196</td>
<td>203 (162, 243)</td>
</tr>
<tr>
<td>AE (Thai)</td>
<td>154</td>
<td>127 (113,141)</td>
</tr>
<tr>
<td>B (Thai)</td>
<td>36</td>
<td>177 (151, 203)</td>
</tr>
<tr>
<td>A/D/AG (Africa)</td>
<td>95</td>
<td>236 (199, 273)</td>
</tr>
<tr>
<td>Overall</td>
<td>756</td>
<td>197 (173, 220)</td>
</tr>
</tbody>
</table>

Although the BED EIA is designed to reduce differences in mean recency periods between different subtypes, the differences still observed may limit utility of this assay.
in populations with divergent subtypes. Such populations may require use of subtype-specific recency periods for the accurate estimation of incidence in.

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 [25]. Test attributes, including reproducibility, intra-run and inter-run coefficient of variation (CV), and inter-operator variability has been well studied by CDC scientists [20] and the manufacturer. These studies indicate that the assay has a very high reproducibility with $R^2 > 0.9$.

**LIMITATIONS OF THE ASSAY**

Classification of individuals by the Sedia™ BED HIV-1 Incidence EIA as recent seroconverters or long-term infections is based on average development of HIV-antibodies calculated from data using a large number of people [1, 21]. However, there are differences among the individuals and the rates at which antibodies are made. 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 assessment of recency of infection. Some people with long-term HIV infections, including AIDS, may be misclassified as recently infected. Such “False Recency Rates” (FRR) have been reported ranging from 0.8 to 16.1% [22]. Persons with diagnosis of AIDS or low CD4+ T cell counts (below 200 cells per μl), recipients of antiretroviral therapy and known “elite controllers” (HIV-infected individuals with known low or undetectable viral loads) should be excluded from the study populations to increase the predictive value of the assay.

**CALCULATING INCIDENCE**

HIV incidence is defined as the number of new HIV infections occurring in a population, usually expressed as a rate of infection per 100 persons per unit time (e.g. “infections per 100 person-years”) [22]. The incidence formula recommended by the U.S. Centers for Disease Control and Prevention, the Office of the Global AIDS Coordinator and the UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance for estimating population level incidence in cross-sectional samples [22, 23, 24] is described below:

$$I_F = \frac{R - (FRR \times P)}{(1-FRR) \omega N} \times 100$$

$I_F$ = annual HIV incidence rate
$N$ = number of HIV-negative samples in the survey
$P$ = number of HIV-positive samples in the survey
$R$ = number of HIV-positive samples testing as recent on the test
$\omega$ = mean duration of recency for the test specified in years
FRR = false recency rate for the test
Estimates of both ω and FRR will carry a degree of uncertainty which will impact the final incidence estimate. Minimum criteria needed to apply this test for incidence estimation includes: 1) a calculation of the local FRR within an acceptable level of precision 2) detection of individuals on ARV in incidence surveys and their exclusion from the incidence analysis; and 3) appropriate sample sizes for incidence surveys and FRR estimation surveys.

Additional guidance and information on how to calculate FRR and sample sizes is available online from UNAIDS/WHO (at http://www.who.int/diagnostics_laboratory/hiv_incidence_may13_final.pdf) [24]. Additional information is available from the CDC, Division of Global HIV-AIDS. Contact Joyce J. Neal (jxn4@cdc.gov) for guidance around study design, implementation, and incidence estimation, Anindya De (AnindyaDe@cdc.gov) for statistical questions, including sample size calculations, and Bharat Parekh (BParekh@cdc.gov) for laboratory related issues. Additional data analysis tools for calculating incidence are available at http://www.sacema.com/page/assay-based-incidence-estimation.

BIBLIOGRAPHY


SYMBOLS AND ABBREVIATIONS
The following symbols appear in Sedia™ BED HIV-1 Incidence EIA product labeling.

- Use by (expiration date)
- Corrosive
- Consult instructions for use
- Temperature limitation (temperature storage)
- Part number
- Temperature limitation (max. temperature storage)
- Lot number (batch code)

Sedia Biosciences Corporation
Portland, Oregon USA
Phone: +1-(503)-459-4159
Fax: +1-(503)-459-4168
Email: customerservice@sediabio.com
Web: www.sediabio.com

Related Products Available from Sedia Biosciences:
SEDIA™ BED HIV-1 Incidence EIA Dried Blood Spot Controls Pack (Cat. No. 1001)
SEDIA™ HIV-1 LAg-Avidity EIA (Cat. No. 1002)
SEDIA™ HIV-1 LAg-Avidity EIA for Dried Blood Spots (Cat. No. 1003)