Dried Blood Spot Control Pack
Calypte® HIV-1 BED Incidence EIA

Supplemental Pack of HIV-1 BED Incidence EIA Controls and Calibrator
Required for the Testing of Dried Serum, Plasma, and Whole Blood Spots

Cat. No. 98133

Contains 4 foil pouches, each containing 5 cards
(20 cards total)

Store at or below -20°C

FOR SURVEILLANCE USE ONLY
Not for diagnostic or clinical use

For use only with the Calypte® HIV-1 BED Incidence EIA

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NAME AND INTENDED USE:
The Calypte® HIV-1 BED Incidence EIA Dried Blood Spot Controls are quality control reagents for use only with the Calypte® HIV-1 BED Incidence EIA test.

The Dried Blood Spot (DBS) Controls should be used only as quality control reagents in place of the liquid controls and Calibrator only when dried serum, dried plasma, or dried whole blood spots are to be tested with the Calypte® HIV-1 BED Incidence EIA test.

If liquid serum or plasma specimens are to be tested, do not use DBS Controls. Instead, use the liquid serum controls that are provided within the Calypte® HIV-1 BED Incidence EIA test.

SUMMARY AND EXPLANATION OF DRIED BLOOD SPOT CONTROLS
The Calypte® HIV-1 BED Incidence EIA Dried Blood Spot Controls are spots of inactivated human serum dried on Schleicher and Schuell/Whatman #903 paper. These controls must be used when dried serum, plasma, or whole blood spots are to be tested with the Calypte® HIV-1 BED Incidence EIA test. For accurate results, test specimens must also be collected on Schleicher and Schuell/Whatman #903 paper.

WARNING: Use of dried controls made by other manufacturers may not produce expected results when used with the Calypte® HIV-1 BED Incidence EIA. Use of the Calypte® HIV-1 BED Incidence EIA Dried Blood Spot Controls in any test other than the Calypte® HIV-1 BED Incidence EIA may not produce accurate results.

MATERIALS PROVIDED
(1) Dried Blood Spot Controls
Twenty (20) cards, each with four (4) color-coded control spots, packaged in four (4) resealable foil pouches with desiccant (5 cards per pouch). Cards are Schleicher and Schuell/Whatman #903 filter paper with HIV-1 BED Incidence EIA serum controls and Calibrator materials immobilized.

Each card consists of one:

• Negative Control (Blue Spot): Inactivated human serum (nonreactive for HBsAg and antibodies to HCV), containing no antibodies to HIV-1 antigens. Contains preservative and blue dye.

• Calibrator (Green Spot): Inactivated human serum (nonreactive for HBsAg and antibodies to HCV), containing a low titer of antibodies to HIV-1 antigens. Contains preservative and green dye.
• Low Positive Control (Yellow Spot): Inactivated human serum (nonreactive for HBsAg and antibodies to HCV), containing a low titer of antibodies to HIV-1 antigens. Contains preservative and yellow dye.

• High Positive Control (Red Spot): Inactivated human serum (nonreactive for HBsAg and antibodies to HCV), containing a high titer of antibodies to HIV-1 antigens. Contains preservative and red dye.

(2) Package Insert

MATERIALS REQUIRED FOR PREPARATION AND USE OF DRIED BLOOD SPOT CONTROLS BUT NOT PROVIDED

• Hole punch, capable of punching 6 mm discs

• Tweezers/forceps


• Multichannel pipette and tips capable of delivering 200 µL

• Reagent Reservoir (VWR International, 800-932-5000, www.vwr.com, catalog number 53504-035) or equivalent

• Biohazardous waste container

• Calypte® HIV-1 BED Incidence EIA (PN #98003) and additional items (which are required but not provided) specified in the Product Insert necessary to perform the test.

• Schleicher and Schuell/Whatman #903 Protein Saver Cards are required for the collection of dried serum, plasma, or blood specimens. (Whatman, Catalog number 10-531-018, 800-245-4024, www.whatman.com)

Note: Additional materials specified in the HIV-1 BED Incidence EIA Product Insert are required to run the EIA
WARNINGS AND PRECAUTIONS
The Calypte® HIV-1 BED Incidence EIA and affiliated components such as the Dried Blood Spot Control Pack are 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.

1. Read this package insert and the package insert for the Calypte® HIV-1 BED Incidence EIA before proceeding. Carefully follow the instructions.
2. Use good laboratory working practices and universal precautions when handling DBS cards. The DBS controls have been inactivated, however, as with any human serum, these should be handled as if capable of transmitting infectious agents.
3. Store Dried Blood Spot Control cards sealed in the original pouch with desiccant at the recommended storage temperature listed on the product.
4. Do not use the Dried Blood Spot control reagents beyond the expiration date printed on the control label.
5. Preparation of the control reagents requires adequate elution time. Follow the directions and do not stop the elution process prior to the time required.
6. Use of Calypte DBS controls in another manufacturer’s assay may not give expected results.
7. Use of another manufacturer’s controls within the Calypte® HIV-1 BED Incidence EIA may not give expected results.
8. If liquid serum or plasma specimens are to be tested, do not use Dried Blood Spot Controls. For the test of liquid specimens (plasma and serum), use the liquid serum controls that are provided within the Calypte® HIV-1 BED Incidence EIA test.
9. Elution of HIV-1 BED Incidence Dried Blood Spot controls and test specimens must be done simultaneously, using the same elution method.
10. For replicate testing of DBS controls and dried specimens, elute an individual disc (6 mm punch) for each replicate.
11. Wipe all 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.
12. Dispose of all unused Dried Blood Spot Control cards, and test specimens as biohazardous waste.
13. For accurate results, dried blood, plasma, and serum test specimens must be collected on Schleicher and Schuell/Whatman #903 filter paper.
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.

Testing of Dried Plasma, Dried Blood, or Dried Serum Spots (DPS, DBS, DSS)

Note: 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 test specimen on the plate. Choose one method below for the preparation of dried controls, Calibrator, and test specimens.

Method A: ELISA Plate Elution of DPS, DBS, or DSS

A-1. Obtain an uncoated non-BED Incidence 96-well ELISA plate.

A-2. Prepare a plate map as described in the Calypte® HIV-1 BED Incidence EIA Product Insert, 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 discs 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 discs 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 with dried plasma, serum and/or whole blood spots and placing the discs into separate wells of the elution plate. The DBS controls can be simultaneously used for testing of punched out discs made from serum, plasma, or whole blood.

A-7. Using a multichannel pipette, add 200µl of specimen diluent to each well that contains a DBS control or specimen disc. 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 plate at 2 to 8°C for 12 to 16 hours.

A-10. Following the elution of the controls and specimens, prepare to run the HIV-1 BED Incidence EIA as described in the EIA Product Insert. Initially, bring all reagents (including the 1x sample diluent and the elution plate) to room temperature.

A-11. The test of dried specimens eluted in an ELISA plate requires a 1:2 dilution. First, use a multichannel pipette to add 50 µL of specimen diluent to each well of the HIV-1 BED Incidence test plate.

A-12. Use a multichannel pipette to mix the contents of 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.


Note: Eluted material can be reused up to 2 days if stored at 2 to 8 ºC. 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 plate.

B-3. Using a 6 mm hole punch, punch out two discs from the DBS Negative Control Spot and place each into separate titertubes consistent with the plate map. The use of titertubes for spot elution may require a minor crimping/folding of the disc 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 discs from the DBS Calibrator Spot and place each into separate titertubes consistent with 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 discs into separate titertubes for elution. The DBS controls can be simultaneously used for testing of punched out discs made from serum, plasma, or whole blood.

B-7. Using a multichannel pipette, add 400µl of specimen 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 clean pipette tips for each control or specimen.
B-8. Cover the titertube rack with a plate sealer or lid.

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

B-10. Following the elution of the controls and specimens, prepare to run the HIV-1 BED Incidence EIA as described in the kit Product Insert. Initially, bring all reagents (including the 1x sample diluent and the elution tubes) to room temperature.

B-11. The test of 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.


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 B-1).

INTERPRETATION OF RESULTS AND ASSAY VALIDITY
The assay validity criteria and calculations for the determination of recent/long term infection in the Calypte® HIV-1 BED Incidence assay are the same for dried and liquid specimens. Please refer to the Calypte® HIV-1 BED Incidence Product Insert for specifications.

BIBLIOGRAPHY


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