Intended Use

The QUANTA Flash Centromere is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of autoantibodies to centromere protein B in human serum on the BIO-FLASH® instrument. This test is to be used in conjunction with clinical findings to aid in assessment of connective tissue diseases including systemic sclerosis.

Summary and Explanation of the Test

Autoantibodies reactive with the centromere region of chromosomes were first described in 1980.1 Traditionally, anti-centromere (CENP) antibodies are detected by indirect immunofluorescence (IIF) using rapidly dividing tissue culture cell lines such as HEp-2 as substrate.1 What is referred to as centromere is now known to consist of several protein subcomponents, the diagnostically most important being CENP-A, CENP-B and CENP-C.1,2 Many of the centromeric subunits have been isolated or cloned. Several other antigens have been identified, but antibodies to these are recognized much less frequently. Most assays for the detection of anti-CENP antibodies are based on recombinant CENP-B1 which have been reported to have a sensitivity of about 95% when compared with IIF using HEp-2 cell substrate.1-3

Centromere antibodies are recognized as a serological marker of a form of systemic sclerosis (SSc) or scleroderma that is commonly referred to as CREST syndrome (calcinosis, Raynaud’s phenomenon, esophageal immotility, sclerodactyly, and telangiectasia).1,2 Patients with anti-centromere antibodies tend to have a more benign form of SSc with less systemic involvement. Antibodies to Centromere proteins are virtually exclusive in samples with anti-RNA Pol III or anti-Scl-70 antibodies.1

Principles of the Procedure

Recombinant centromere protein B is coated on to paramagnetic beads, which are stored in the reagent cartridge under conditions that preserve the antigen in its reactive state. When the assay cartridge is ready to be used for the first time, a buffer solution is added to the tube containing the preserved beads, and the beads are mixed with the buffer. The reagent cartridge is then loaded onto the BIO-FLASH® instrument.

A patient serum sample is prediluted by the instrument using system rinse added to a disposable plastic cuvette. Small amounts of the diluted patient serum, the CENP-B beads, and the assay buffer are all combined into a second cuvette, and mixed. This cuvette is incubated at 37°C. The beads are then magnetized and washed several times. Isoluminol conjugated antibody is then added to the cuvette, and incubated at 37°C. Again, the beads are magnetized and washed repeatedly. The isoluminol conjugate produces a luminescent reaction when reagents (“Triggers”) are added to the cuvette. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. The RLU are proportional to the amount of bound isoluminol conjugate, which in turn is proportional to the amount of anti-CENP antibodies bound to the CENP-B on the beads.

The QUANTA Flash Centromere assay utilizes a predefined lot specific Master Curve that is uploaded into the instrument through the reagent cartridge barcode. Based on the results of running two calibrators, an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the RLU obtained for each patient.
Reagents

1. QUANTA Flash Centromere reagent cartridge contains the following reagents for 50 determinations:
   a. Centromere coated paramagnetic beads, preserved prior to first time use.
   b. Assay buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
   c. Tracer IgG– Isoluminol labeled anti-human IgG antibodies, containing buffer, protein stabilizers and preservative.

2. Resuspension buffer, 1 vial - colored pink, containing buffer, protein stabilizers and preservatives.

Warnings

1. The assay buffer contains a chemical (0.02% chloramphenicol) known to the State of California to cause cancer.
2. Sodium azide is used as a preservative. Sodium azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. Use appropriate personal protective equipment while working with the reagents provided.
4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for In Vitro Diagnostic Use.
2. This assay is only for use in the BIO-FLASH instrument.
3. Strict adherence to the resuspension protocol is recommended.
4. Once opened, this reagent cartridge must be stored in the instrument’s reagent carousel. Care should be taken to avoid spilling the reagents when the reagent cartridge is first placed into the instrument.
5. Chemical contamination of the reagents can result from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BIO-FLASH operator’s manual.

Storage Conditions

1. Store unopened reagent cartridges and resuspension buffer at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Opened reagent cartridges should be stored onboard the instrument and are stable for a total of 56 days, after which time they must be discarded. The BIO-FLASH software monitors the expiration dates of the onboard cartridges, as well as the reagent cartridge lots.
Specimen Collection

This procedure should be performed with a serum specimen. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Specimens containing up to 10 mg/dL bilirubin, 200 mg/dL hemoglobin, 1000 mg/dL triglycerides, 224 mg/dL cholesterol, or 500 IU/mL IgM rheumatoid factor did not show interference in the QUANTA Flash Centromere.

Following collection, the serum should be separated from the clot. CLSI Document H18-A4 recommends the following storage conditions for samples.

1. Store samples at room temperature no longer than 8 hours.
2. If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C.
3. If the assay will not be completed within 48 hours, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Procedure

Materials Provided

1. QUANTA Flash Centromere Reagent Cartridge
2. Resuspension buffer
3. Transfer pipet

Additional Materials Required But Not Provided

- BIO-FLASH instrument with operating computer
- BIO-FLASH System Rinse (Part Number: 3000-8205)
- BIO-FLASH Triggers (Part Number: 3000-8204)
- BIO-FLASH Cuvettes (Part Number: 3000-8206)
- QUANTA Flash Centromere Calibrators (Part Number: 701196)
- QUANTA Flash Centromere Controls (Part Number: 701197)

Using the BIO-FLASH Chemiluminescent Analyzer

1. Refer to the operator’s manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software. For additional information and for troubleshooting problems with this assay, contact INOVA Diagnostics, Inc. technical service at the address or telephone number found at the end of this Direction Insert.
2. To empty the solid waste container, open the waste drawer. Remove the solid waste container and dispose of the used cuvettes properly. Replace the solid waste container, close the waste drawer, and click Yes in the Empty Waste Drawer window.
3. To replace the triggers, click the Bulks Inventory F9 button (upper right).
   a. In the Inventory – Bulks screen, click the Triggers button on the left. A new window will pop up titled Add Triggers – Remove old bottles.
b. Open and remove the waste drawer on the BIO-FLASH instrument. Dispose of any cuvettes in the dry waste drawer. Click Yes on the Empty Waste Drawer window. Remove the trigger bottles from their holders and click the Next button. Unscrew the old trigger bottles from their caps and replace with new triggers. Be sure to do them one at a time, and match the color-coded caps (white to white and red to red).

c. Follow the instructions in the new window Add Triggers – Add Trigger 2 bottle. Once the barcode has been accepted, place Trigger 2 into the color-coded white holder. Click Next.

d. Follow the instructions in the window Add Triggers – Add Trigger 1 bottle. Once the barcode has been accepted, place Trigger 1 into the color-coded red holder. Click Finish. Replace and close the waste drawer.

4. To replace the System Rinse container, click the Bulks Inventory F9 button (upper right corner). In the Inventory – Bulks screen, click the Sys. Rinse button. In the new window Add System Rinse – Remove bottles, click Next. Follow the instructions in the new window Add System Rinse – Add bottle. Once the barcode has been accepted, click Finish if necessary.

5. To empty the Fluid Waste Container, from the Inventory – Bulks screen, click the Fluid Waste button. Remove and dispose of the fluid waste. Click Next. Once the empty bottle has been replaced, click Finish.
Method

Reagent Cartridge Preparation
The first time the reagent cartridge is to be used, the storage seals on the reagent tubes must be pierced, and the Centromere coated beads must be mixed with resuspension buffer. Note: Do not use the reagent cartridge if any signs of damage are observed.

1. Place the reagent cartridge on a solid surface. Hold the reagent cartridge in place with one hand. With your other hand, firmly grasp the red pull-tab on the back of the reagent cartridge and pull it out completely.

2. Press the two tabs on the sides of the piercing cap (grey part) and apply pressure to the top portion of the reagent cartridge until it snaps down into a locked position. The tabs should no longer be visible.

3. Resuspend the kit reagents:
   a. Uncap the resuspension buffer vial and collect fluid into the transfer pipette provided. The entire contents of the vial will be used.
   b. Slide the door in the reagent cartridge lid to the open position by gently pressing the narrow side on the reagent cartridge, and hold it in this position. Analytically transfer the entire contents of the vial into the bead reagent tube through the one single hole on the top of the reagent cartridge.
   c. Mix the contents of the bead reagent tube by aspirating and dispensing the liquid at least 30 times. If visible clumps of beads are observed, continue to mix the solution for another 30 times. If the microparticles do not resuspend, DO NOT USE THE CARTRIDGE.
   d. Be sure to dispense all the liquid before removing the pipette from the tube and discarding it.

4. Peel the sticker off the top of the reagent cartridge to reveal the other three holes.

5. Place the reagent cartridge into any open slot on the reagent carousel of the BIO-FLASH instrument. Once the cartridge is placed into the reagent carousel, the instrument performs additional periodic mixing of the beads.
**Assay Calibration**

1. Each new lot of reagent cartridge must be calibrated prior to first time use. The software will not allow a new lot to be used until it is calibrated.

2. Refer to the section titled **QUANTA Flash® Centromere Calibrators 701196** of this Direction Insert for detailed instructions of how to calibrate the reagent cartridge.

3. Once the calibration is validated, the reagent cartridge lot on which the calibration was performed is ready for use.

**Programming and Running Samples**

1. Press the **Worklist** button at the top of the screen and select the **Racks** tab at the bottom.

2. Select the sample rack to be used by highlighting the rack on the screen or by scanning its barcode with the handheld barcode reader. Scan or type in the sample name, select the sample type, container type (tube/cup) and select Centromere from the assay panel. Repeat these steps for all samples.

3. Load the samples into the selected positions in the sample rack, and load the rack into the sample carousel of the instrument.

4. If all required materials are onboard the instrument, the start icon will be available, in green, at the top of the screen. Press the start icon to begin the run.

**Quality Control**

The **QUANTA Flash Centromere Controls** (sold separately - INOVA Item Number 701197) contains both Centromere Positive and Negative Controls. Refer to the section titled **QUANTA Flash® Centromere Controls 701197** of this Direction Insert for detailed instructions on how to input the unit value and standard deviation of each control into the software, as well as how to run the controls. Controls are recommended to be run once every day that the assay is used; however, users should also consider national/local regulatory requirements.

**Calculation of Results**

A six point Master Curve is produced at INOVA for each new lot of QUANTA Flash Centromere. This four parameter logistic curve is encoded in the barcode of each reagent cartridge. Once a reagent cartridge has been calibrated, a machine specific working curve will be used to convert the RLU to CU. The antibody reactivity for Centromere can then be classified according to the table below.

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>CU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Positive</td>
<td>≥20</td>
</tr>
</tbody>
</table>

Reactivity in CU is directly related to the titer of the autoantibody in the patient sample. Increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in CU, which is proportional to the amount of antibody.

The analytical measuring range (AMR) of the assay is 3.4 CU to 708.9 CU. If a patient result is less than 3.4 CU, then the BIO-FLASH system will report it as “<3.4 CU”. Since this is less than 20 CU, it is considered a negative result. If a patient result is greater than 708.9 CU, then the BIO-FLASH system will report it as “>708.9 CU”. This is considered a positive result. The BIO-FLASH software has an Auto-Rerun option available. If this option is selected, the instrument will automatically rerun any sample that has a result >708.9 CU by further diluting it by a factor of 20, and calculate the actual CU using this additional dilution factor.
Interpretation of Results

The QUANTA Flash Assay is capable of detecting small differences in patient populations. Each laboratory should establish its own normal range based upon its own controls and patient population according to their own established procedures.

It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the INOVA QUANTA Flash Centromere chemiluminescent immunoassay. Values obtained with different manufacturers’ assay methods may not be used interchangeably. The magnitude of the reported antibody levels cannot always be correlated to an endpoint titer.”

Limitations of the Procedure

1. Not all patients with systemic sclerosis are positive for anti-CENP-B antibodies.
2. Results of this assay should be used in conjunction with clinical findings and other serological tests.
3. Failure to adequately resuspend the Centromere coated beads may yield lower values than if the beads are properly resuspended.
4. The performance characteristics of this assay have not been established for matrices other than serum.

Expected Values

To establish the expected values, sera from 400 apparently healthy blood donors were tested. The average was <3.4 CU and the 95% was calculated as <3.4 CU.

Clinical Sensitivity and Specificity

The clinical validation study included 137 samples from patients with systemic sclerosis (SSc), 50 rheumatoid arthritis, 137 systemic lupus erythematosus, 49 inflammatory bowel disease, 27 Crohn’s disease, 30 ulcerative colitis, 19 multiple sclerosis, 31 hepatitis B virus, 24 hepatitis C virus, 8 human immunodeficiency virus, 9 syphilis and 37 patients with other diseases. In addition specimens from 400 normal blood donors were included.

The results of this testing are shown below:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Analysis (95% confidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSc</td>
</tr>
<tr>
<td>QUANTA Flash®</td>
<td></td>
</tr>
<tr>
<td>Centromere</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
</tr>
<tr>
<td>Negative</td>
<td>105</td>
</tr>
<tr>
<td>Total</td>
<td>137</td>
</tr>
</tbody>
</table>

* 5 samples were from apparently healthy individuals, one sample from MCTD patient, one sample from an incomplete CREST patient (CREST), one sample from a SLE patient; 7/8 samples were also positive by ELISA
Method Comparison with Predicate Device

Samples for method comparison analysis included samples from the clinical validation studies and from analytical comparison. These samples were tested on both the QUANTA Flash Centromere and on the predicate ELISA.

<table>
<thead>
<tr>
<th>Method Comparison (N=361)</th>
<th>Centromere ELISA</th>
<th>Percent Agreement (95% Confidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>QUANTA Flash® Centromere CIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>74</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>13*</td>
<td>273</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>274</td>
</tr>
</tbody>
</table>

* three samples were from SSc patients and 8 samples from patients with SLE; two with unknown diagnosis
# MCTD patient with Raynaulds Syndrome

Precision and Reproducibility

Precision of the QUANTA Flash Centromere assay was evaluated by running 8 patients in accordance with CLSI EP5-A2, and the data are summarized below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean (CU)</th>
<th>SD</th>
<th>% CV</th>
<th>SD</th>
<th>% CV</th>
<th>SD</th>
<th>% CV</th>
<th>SD</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>80</td>
<td>6.2</td>
<td>0.2</td>
<td>2.6%</td>
<td>0.2</td>
<td>3.0%</td>
<td>0.3</td>
<td>5.4%</td>
<td>0.4</td>
<td>6.7%</td>
</tr>
<tr>
<td>B</td>
<td>80</td>
<td>13.5</td>
<td>0.5</td>
<td>3.7%</td>
<td>0.3</td>
<td>2.1%</td>
<td>0.7</td>
<td>4.9%</td>
<td>0.9</td>
<td>6.5%</td>
</tr>
<tr>
<td>C</td>
<td>80</td>
<td>22.9</td>
<td>0.9</td>
<td>3.7%</td>
<td>0.5</td>
<td>2.2%</td>
<td>0.9</td>
<td>4.0%</td>
<td>1.4</td>
<td>5.9%</td>
</tr>
<tr>
<td>D</td>
<td>80</td>
<td>60.9</td>
<td>2.0</td>
<td>3.2%</td>
<td>1.3</td>
<td>2.1%</td>
<td>3.4</td>
<td>5.5%</td>
<td>4.1</td>
<td>6.7%</td>
</tr>
<tr>
<td>E</td>
<td>80</td>
<td>103.3</td>
<td>5.7</td>
<td>5.6%</td>
<td>0.4</td>
<td>0.4%</td>
<td>4.2</td>
<td>4.1%</td>
<td>7.1</td>
<td>6.9%</td>
</tr>
<tr>
<td>F</td>
<td>80</td>
<td>197.9</td>
<td>13.2</td>
<td>6.7%</td>
<td>0.0</td>
<td>0.0%</td>
<td>11.9</td>
<td>6.0%</td>
<td>17.7</td>
<td>9.0%</td>
</tr>
<tr>
<td>G</td>
<td>80</td>
<td>321.4</td>
<td>24.5</td>
<td>7.6%</td>
<td>0.0</td>
<td>0.0%</td>
<td>27.8</td>
<td>8.7%</td>
<td>37.1</td>
<td>11.5%</td>
</tr>
<tr>
<td>H</td>
<td>80</td>
<td>463.2</td>
<td>33.6</td>
<td>7.3%</td>
<td>0.0</td>
<td>0.0%</td>
<td>22.9</td>
<td>4.9%</td>
<td>40.7</td>
<td>8.8%</td>
</tr>
</tbody>
</table>

Limits of Detection; Linear and Analytical Measuring Ranges

The lower limit of detection of this assay according to CLSI EP17-A is approximately 370.2 RLU, which is equivalent to 1.0 CU, which is well below the bottom of the AMR. The upper limit of detection is about 774,000 RLU, which is approximately 39% above the top of the AMR. The entire AMR, from 3.4 CU to 708.9 CU, is linear. A linearity study was performed according to CLSI EP6-A and the data is summarized below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test Range (CU)</th>
<th>Slope (95% CI)</th>
<th>Y-intercept (95% CI)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.77 - 17.7</td>
<td>0.94 (0.91 to 0.97)</td>
<td>1.01 (0.69 to 1.33)</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>6.3 - 63.7</td>
<td>0.98 (0.96 to 1.00)</td>
<td>1.89 (1.15 to 2.64)</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>9.33 - 93.26</td>
<td>0.98 (0.96 to 1.01)</td>
<td>2.45 (1.16 to 3.74)</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>12.74 - 127.4</td>
<td>0.99 (0.97 to 1.01)</td>
<td>2.27 (0.89 to 3.64)</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>24.44 - 244.4</td>
<td>0.99 (0.97 to 1.01)</td>
<td>-0.20 (-3.18 to 2.78)</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>113.59 - 1135.9</td>
<td>0.99 (0.95 to 1.03)</td>
<td>-39.07 (-67.38 to -10.76)</td>
<td>0.99</td>
</tr>
</tbody>
</table>
QUANTA Flash® Centromere 701196

Calibrators
For In Vitro Diagnostic Use

Intended Use
The QUANTA Flash Centromere Calibrators are intended for use with the QUANTA Flash Centromere chemiluminescent immunoassay (CIA) on the BIO-FLASH® instrument. Each calibrator establishes a point of reference for the working curve that is used to determine Chemiluminescent Unit (CU) values in the measurement of Centromere antibodies in serum.

Summary and Principles of the Procedure
The QUANTA Flash Centromere CIA utilizes a predefined lot specific Master Curve that is stored in the reagent cartridge barcode. The QUANTA Flash Centromere Calibrators are designed to produce an instrument specific Working Curve from the parameters of the Master Curve, with a decision point based on the performance characteristics and clinical evaluation of the QUANTA Flash Centromere CIA. Calibrators are tested on multiple instruments with multiple lots of reagents prior to value assignment.

Reagents
1. QUANTA Flash Centromere Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL, prediluted, ready to use reagent. Calibrators contain human antibodies to centromere in buffer, stabilizers, and preservatives.
2. QUANTA Flash Centromere Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL, prediluted, ready to use reagent. Calibrators contain human antibodies to centromere in buffer, stabilizers, and preservatives.

Warnings
1. The calibrators contain a chemical (0.02% chloramphenicol) known to the State of California to cause cancer.
2. Sodium azide is used as a preservative. Sodium azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the QUANTA Flash Centromere Calibrators should be handled in the same manner as potentially infectious material.¹
4. Use appropriate personal protective equipment while working with the reagents provided.
5. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions
1. This product is for In Vitro Diagnostic Use.
2. The QUANTA Flash Centromere Calibrators are for use with the QUANTA Flash Centromere
3. Do not transfer the calibrator reagents to secondary tubes. The barcodes on the tubes are used by the instrument to match the calibrators to the proper assay type.

4. Once a calibrator tube is opened, it is good for up to 8 hours or 4 calibrations, after which the reagent must be discarded.

5. Chemical contamination of the reagents can result from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BIO-FLASH operator’s manual.

**Storage Conditions**

1. Store unopened calibrators at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.

2. Opened calibrators must be discarded after 8 hours.

**Procedure**

a. Each new lot of reagent cartridge must be calibrated prior to first time use. The software will not allow a new lot to be used until it is calibrated.

2. Each calibrator must be gently mixed before use to insure homogeneity. Avoid foam formation, as bubbles may interfere with the instruments liquid level detection. Uncap each calibrator tube and place both into a sample rack, with the barcodes facing forward through the gaps in the rack. Place the sample rack into the sample carousel of the BIO-FLASH instrument, and close the door. The instrument will read the barcodes on the calibrator tubes, and identify the required reagent cartridge. Refer to the operator’s manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software.

3. The instrument will run each calibrator in triplicate. After the Calibrators have been run, the software will require the calibration to be validated. From the Instrument Summary screen, click the Choose more options – Ctrl-M (▼) arrow button. Select Calibration Ctrl-F3. In the Calibration window, highlight the desired assay, and click Details.

4. In the new Calibration Details window, select the calibration that was just performed. The Master Curve appears as a dashed line, while the new Working Curve appears as a solid line. If the calibration results are valid, a validation button will appear in the lower left of the screen. Click the Validate Calibration button.

5. Once the calibration is validated, the reagent cartridge lot on which the calibration was performed is ready for use. It is recommended that the QUANTA Flash Centromere Controls (sold separately – part number 701197) be run after a reagent cartridge lot is calibrated.

**Traceability**

No international reference serum for anti-Centromere antibodies is available that allows for the standardization of anti-Centromere antibody assays. Instead the Reference serum from the Center of Disease Control and Prevention for anti-Centromere antibodies has been tested (IS2134 ANA #8 Centromere pattern FANA) and a concentration of 500.8 CU has been determined.

**Limitations**

These calibrators are designed for 4 calibrations. The total time the calibrator tubes can be uncapped onboard the instrument is 8 hours. If the calibrators are left uncapped, onboard, for any longer period of time, they should be discarded. Using the same calibrator tubes for more than 4 calibrations and/or more than 8 hours can result in improper calibration of the assay, which in turn
could give erroneous results.
Controls
For In Vitro Diagnostic Use

Intended Use
The QUANTA Flash Centromere Controls are intended for quality control purposes of the QUANTA Flash Centromere chemiluminescent immunoassay (CIA) kit run on a BIO-FLASH® instrument.

Summary and Principles of the Procedure
The QUANTA Flash Centromere Controls are made up of a Negative Control and a Positive Control. Each contains a different amount of Centromere antibodies. The Negative Control is designed to assess precision and accuracy of the assay at very low antibody levels. The Positive Control is designed to assess precision and accuracy of the assay at moderate to high antibody levels.

Reagents
1. QUANTA Flash Centromere Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to centromere in buffer, stabilizers, and preservatives.
2. QUANTA Flash Centromere Positive Control: Two (2) barcode labeled tubes containing 0.5mL, ready to use reagent. Controls contain human antibodies to centromere in buffer, stabilizers, and preservatives.

Warnings
1. The controls contain a chemical (0.02% chloramphenicol) known to the State of California to cause cancer.
2. Sodium azide is used as a preservative. Sodium azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the QUANTA Flash Centromere Controls should be handled in the same manner as potentially infectious material.5
4. Use appropriate personal protective equipment while working with the reagents provided.
5. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions
1. This product is for In Vitro Diagnostic Use.
2. The QUANTA Flash Centromere Controls are for use with the QUANTA Flash Centromere assay.
3. Do not transfer the control reagents to secondary tubes. The barcodes on the tubes are used by the instrument to identify the control.
4. Once opened, each control tube is good for up to 15 uses with a maximum time of 10 minutes onboard the instrument per use.

5. Chemical contamination of the reagents can result from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BIO-FLASH operator’s manual.

**Storage Conditions**

1. Store unopened controls at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.

2. Opened controls can be used for up to 15 times, with a maximum time of 10 minutes onboard the instrument per use. The total time the control tubes can be uncapped onboard the instrument is 2 ½ hours, or 10 minutes per use. If the controls are left uncapped, onboard, for a total time greater than 2 ½ hours, they should be discarded. Using the same control tube for more than 15 uses and/or more than 2 ½ hours total, can result in erroneous results.

3. For optimal stability, remove controls from the system immediately after sampling and store them at 2-8°C capped in the original vial.

**Procedure**

**To Create New QC Materials for the Centromere Assay**

1. Prior to using QUANTA Flash Centromere Controls for the first time on the instrument, enter the name, lot, expiration, value (or dose), and target SD information into the software.

2. From the Instrument Summary screen, click the Choose more options – Ctrl-M (▼) arrow button. Select QC Ctrl-F2. Click the New QC Material button.

3. A lot specific data sheet is included with each Control set. First enter the name, lot number, expiration from this data sheet into the software. Next, click the Add Assay button. In the new window, make sure the Show All Assays box is checked. Select the Centromere assay from the list and click Add. Finally, enter in the target dose and target SD. Click Save. Perform this process for both controls.

**To Create a New Lot for Existing QC Materials**

1. Prior to using a new lot of QUANTA Flash Centromere Controls for the first time, enter the lot, expiration, value (or dose), and target SD information into the software.

2. From the Instrument Summary screen, click the Choose more options – Ctrl-M (▼) arrow button. Select QC Ctrl-F2. Highlight the CEN assay in the column on the left. Then highlight the appropriate control material on the right (either “CENN” for the Negative Control or “CENP” for the Positive Control). Click the New QC Lot button.

3. A lot specific data sheet is included with each Control set. Enter the information from this data sheet into the software. This should include the lot number, expiration, target dose, and target SD. If necessary, click the Add Assay button. In the new window, make sure the Show All Assays box is checked. Select the CEN assay from the list and click Add. Click Save. Perform this process for both controls.

It is recommended that the QUANTA Flash Centromere Controls be run once each day that the assay will be used; however users should also consider national/local regulatory requirements.
Each control must be gently mixed before use to insure homogeneity. Avoid foam formation, as bubbles may interfere with the instrument's liquid level detection. Uncap each control tube and place both into a sample rack, with the barcodes facing forward through the gaps in the rack. Place the sample rack into the sample carousel of the BIO-FLASH instrument, and close the door. The instrument will read the barcodes on the control tubes, and identify the required reagent cartridge. Refer to the operator’s manual provided with the BIO-FLASH system for detailed operating instructions of the BIO-FLASH chemiluminescent analyzer and the BIO-FLASH software.

Traceability
No international reference serum for anti-Centromere antibodies is available that allows for the standardization of anti-Centromere antibody assays. Instead the Reference serum from the Center of Disease Control and Prevention for anti-Centromere antibodies has been tested (IS2134 ANA #8 Centromere pattern FANA) and a concentration of 500.8 CU has been determined.

Limitations
These controls are designed for 15 uses. The label of each control tube has a row of 15 boxes that may be checked off so as to track the number of uses. The total time the control tubes can be uncapped onboard the instrument is 2 ½ hours, or 10 minutes per use. If the controls are left uncapped, onboard, for any longer period of time, they should be discarded. Using the same control tubes for more than 15 uses and/or more than 2 ½ hours total, can result in erroneous results.
References


Symbols Used

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<th>Symbol</th>
<th>Description</th>
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<td>IVD</td>
<td>In Vitro diagnostic medical device</td>
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<td>i</td>
<td>Consult instructions for use</td>
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<tr>
<td>℃</td>
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