QUANTA Flash® HMGCR Reagents

For *In Vitro* Diagnostic Use. CLIA Complexity: Moderate

REF **701333** **Rx Only**

Intended Use

QUANTA Flash HMGCR is a chemiluminescent immunoassay for the semi-quantitative determination of IgG autoantibodies against HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) antigen in human serum. The presence of anti-HMGCR antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathy (IIM).

Summary and Explanation of the Test

Autoantibodies to 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) are found in a subgroup of patients with idiopathic inflammatory myopathies (IIM)[1]. In the original study that identified these autoantibodies, 16/17 antibody positive patients had immune mediated necrotizing myopathies (IMNM)[2]. The presence of anti-HMGCR is associated, but not limited to statin exposure.

Recently, a large international multi-center study on anti-HMGCR antibodies was performed. The highest prevalence of anti-HMGCR antibodies was found in patients with IMNM (31/69, 44.9%) followed by juvenile dermatomyositis (JDM) (3/45, 6.7%), polymyositis (PM) (18/406, 4.4%), and dermatomyositis (DM) (10/525, 1.9%). Only two samples from the disease comparator controls [one primary Sjögren's syndrome (pSS) and one systemic lupus erythematosus (SLE)] were positive for anti-HMGCR antibodies resulting in high disease specificity of 99.7%. Of all anti-HMGCR antibody positive patients, 31 (48.4%) had a diagnosis of IMNM, 18 (28.1%) had polymyositis (PM), 10 (15.6%) had dermatomyositis (DM), 3 (4.7%) had juvenile DM (JDM), one (1.6%) had primary Sjögren`s syndrome, and one (1.6%) had systemic lupus erythematosus[3].

Principles of the Procedure

HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) antigen is coated on to paramagnetic beads, which are stored in the reagent cartridge lyophilized. When the assay cartridge is ready to be used for the first time, a buffer solution is added to the tube containing the beads, and the beads are resuspended with the buffer. The reagent cartridge is then loaded onto the BIO-FLASH instrument.

A patient serum sample is diluted 1:17 by the instrument in a disposable plastic cuvette. An aliquot of the diluted patient serum, HMGCR-coupled beads, and assay buffer are 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 anti-human IgG 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 “Trigger” reagents are added to the cuvette. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. RLU values are proportional to the amount of bound isoluminol conjugate, which in turn is proportional to the amount of anti-HMGCR antibodies bound to the antigen on the beads.

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

Reagents

1. QUANTA Flash HMGCR Reagent Cartridge contains the following reagents for 50 determinations:
    a. HMGCR coated paramagnetic beads, lyophilized.
    b. Assay buffer – colored pink, containing protein stabilizers and preservatives.
    c. Tracer IgG – Isoluminol labeled anti-human IgG antibody, containing buffer, protein stabilizers and preservative.
2. Resuspension buffer, 1 vial - colored clear, containing buffer, protein stabilizers and preservatives.

Warnings

1. 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.
2. Use appropriate personal protective equipment while working with the reagents provided.
3. 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. The BIO-FLASH software monitors the onboard (in-use) expiration as well as the reagent lot expiration (shelf-life) of the reagent cartridge. The system will not allow use of a cartridge which has expired.

Specimen Collection, Preparation and Handling

This procedure should be performed on a serum specimen. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Samples containing up to 1 mg/mL bilirubin, 2 mg/mL hemoglobin, 1000 mg/dL triglycerides, 333 mg/dL cholesterol, 35 mg/mL IgG, 153 IU/mL IgM rheumatoid factor, 600 ng/mL atorvastatin, 600 ng/mL simvastatin, 0.72 mg/mL coenzyme Q, 24 µg/mL pyrroloquinoline quinone or 36 µg/mL methylprednisolone did not show interference in the QUANTA Flash HMGCR assay.

Following collection, the serum should be separated from the clot. The following storage conditions for samples are recommended:

1. Samples can be stored at room temperature for up to 48 hours.
2. Samples can be stored at 2-8°C for up to 14 days.
3. If the assay will not be completed within 14 days, or for shipment of the sample, freeze at -20°C or lower. Samples may be frozen and thawed up to 3 times. Frozen samples must be mixed well after thawing and prior to testing.

Procedure

Materials Provided

1 QUANTA Flash HMGCR Reagent Cartridge

1 Resuspension buffer

1 Transfer pipette

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 HMGCR Calibrators (Part Number: 701331)

QUANTA Flash HMGCR Controls (Part Number: 701332)

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.

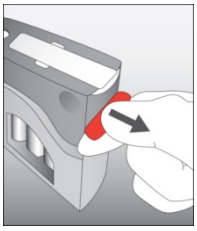
5. To empty the Fluid Waste Container, from the **Inventory – Bulks** screen, click the **Fluid Waste** button. Remove and dispose of the fluid waste. Click **Next**. Once the empty bottle has been replaced, click **Finish**.

Method

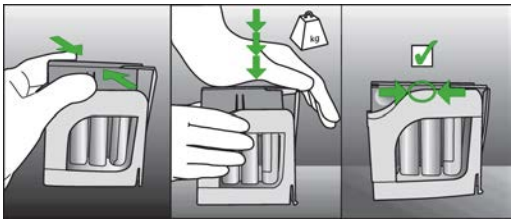
Reagent Cartridge Preparation

The first time the reagent cartridge is to be used, the following steps must be followed to accurately install the cartridge onto the BIO-FLASH instrument. Note: Do not use the reagent cartridge if any signs of damage are observed.

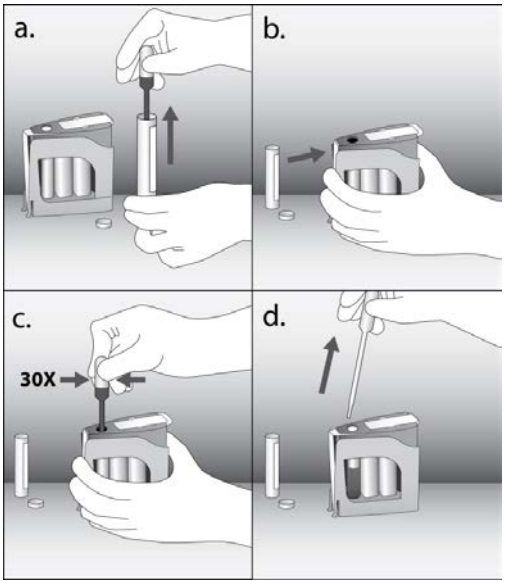
1. Place the reagent cartridge on a solid surface. Hold the reagent cartridge in place with one hand. With your other hand, firmly grasp the red pull-tab on the back of the reagent cartridge and pull it out completely.



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. DO NOT INVERT THE OPEN CARTRIDGE.



3. Resuspend the HMGCR microparticles:
   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. Carefully transfer the entire contents of the vial into the microparticle reagent tube through the one single hole on the top of the reagent cartridge.
   c. Mix the contents of the microparticle 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.

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® HMGCR Calibrators 701331** 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 HMGCR 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 F4** icon to begin the run.

Quality Control

The QUANTA Flash HMGCR Controls (sold separately - Inova Item Number 701332) contains both HMGCR Positive and Negative Controls. Refer to the section titled **QUANTA Flash® HMGCR Controls 701332** of the Direction Insert for detailed instructions on how to input all required information 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 Master Curve is created at Inova for each new lot of QUANTA Flash HMGCR. The parameters of the curve are encoded in the barcode of each reagent cartridge. During calibration, an instrument specific working curve is created based on the Master Curve, and is used to convert the RLU values to CU. The antibody reactivity to HMGCR can then be classified according to the table below.

Reactivity	CU
Negative	<20
Positive	≥20

Reactivity in CU is directly related to the titer of the autoantibody in the patient sample. Increases and decreases in patient autoantibody 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 1.5 CU to 550.0 CU. If a patient result is less than 1.5 CU, the BIO-FLASH system will report it as “<1.5 CU”. Since this is less than 20 CU, it is considered a negative result. If a patient result is greater than 550.0 CU, the BIO-FLASH system will report it as “>550.0 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 >550.0 CU after additional 20 fold dilution, thereby bringing the measured value within the AMR. The final result will be calculated by the software by taking into account the additional dilution factor. As the highest value that can be measured is 550.0 CU, the highest value that can be reported is 11000 CU.

Interpretation of Results

Each laboratory is advised to verify the manufacturer provided reference range, and may 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 HMGCR chemiluminescent immunoassay. Values obtained with different manufacturers’ assay methods must not be used interchangeably.”

Limitations of the Procedure

1. Not all idiopathic inflammatory myopathy patients are positive for HMGCR antibodies.
2. Results of this assay should be used in conjunction with clinical findings and other serological tests.
3. Failure to adequately resuspend the HMGCR 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.

Cut-off (Reference Range)

The assay cut-off was determined using 145 samples from reference subjects consisting of 23 apparently healthy blood donors, 22 patients with infectious diseases, 18 systemic sclerosis patients, 20 systemic lupus erythematosus patients, 48 end stage renal disease patients and 14 other samples. The cut-off was established based on the 99th percentile of the results obtained on the reference subjects. The cut-off was assigned a value of 20 CU.

Expected Values

The expected result for normal population is negative. Anti-HMGCR antibody levels were analyzed using the QUANTA Flash HMGCR on a panel of 100 apparently healthy blood donors (50 females/50 males, ages 17 to 57 years, with an average and median age of 34 years). With a cut-off of 20 CU, all samples were negative with the QUANTA Flash HMGCR. The mean concentration was 1.8 CU and the values ranged from <1.5 to 8.0 CU.

Traceability

No international standard serum for anti-HMGCR antibodies is available that allows for the standardization of anti-HMGCR antibody assays.

Clinical Sensitivity and Specificity

A total of 723 samples were used in the clinical validation study, including 257 samples from IIM patients, and 466 control samples from patients with other autoimmune syndromes and various infectious diseases.

Distribution of samples and anti-HMGCR antibody positivity rate in the validation study:

Patient Group	**N**	**N Positive**	**% Positive**
Hepatitis B Virus (HBV)	14	0	0.0%
Hepatitis C Virus (HCV)	13	0	0.0%
Human Immunodeficiency Virus (HIV)	13	0	0.0%
Syphilis	12	0	0.0%
Systemic lupus erythematosus (SLE)	80	0	0.0%
Sjögren's syndrome (SS)	44	0	0.0%
Scleroderma	59	1	1.7%
Mixed Connective Tissue Disease	36	0	0.0%
Celiac	25	0	0.0%
Rheumatoid Arthritis	39	0	0.0%
Fibromyalgia	13	0	0.0%
Hypothyroidism	14	0	0.0%
Lyme Disease	15	0	0.0%
Polymyalgia Rheumatica	13	0	0.0%
Primary Raynaud's Syndrome	15	0	0.0%
Sarcoidosis	15	0	0.0%
Breast Cancer	10	0	0.0%
Colorectal Cancer	10	0	0.0%
Lung Cancer	10	0	0.0%
Ovarian Cancer	10	0	0.0%
Paraneoplastic Syndrome	6	0	0.0%
Total Controls	**466**	**1**	**0.2%**
Dermatomyositis (DM)	67	0	0.0%
Amyopathic Dermatomyositis	8	0	0.0%
Juvenile Dermatomyositis	13	1	7.7%
Polymyositis (PM)	88	9	10.2%
Inclusion Body Myositis	13	0	0.0%
Overlap	1	0	0.0%
Immune Mediated Necrotizing Myopathy (IMNM)	67	55	82.1%
Total idiopathic inflammatory myopathy (IIM)	**257**	**65**	**25.3%**
Total	**723**	-	-

Clinical performance (sensitivity and specificity) of the QUANTA Flash HMGCR were analyzed in the table below:

Clinical Analysis (N=723)		QUANTA Flash HMGCR			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	IIM	65	192	257	Sensitivity: 25.3% (20.4 – 30.9%)
	Controls	1	465	466	Specificity: 99.8% (98.8 – 100.0%)
	Total	66	657	723	

Precision and Reproducibility

The precision of the QUANTA Flash HMGCR assay was evaluated on 7 samples containing various concentrations of HMGCR antibodies in accordance with CLSI EP5-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline. Samples were run in duplicates, twice a day, for 20 days. Repeatability (Within run), between run, between day and within-laboratory precision (total precision) were calculated and are summarized in the table below.

QUANTA Flash HMGCR			**Repeatability**		**Between-Run**		**Between-Day**		**Within-Laboratory**	
Sample ID	**N**	**Mean (CU)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**
1	80	10.9	0.5	4.6	0.2	2.2	0.5	4.3	0.7	6.7
2	80	48.3	1.1	2.4	1.5	3.1	2.6	5.3	3.2	6.6
3	80	16.3	0.3	2.0	0.5	3.4	0.8	5.0	1.0	6.3
4	80	23.4	0.4	1.9	0.9	3.8	0.9	4.1	1.4	5.9
5	80	76.2	2.1	2.8	1.7	2.2	4.0	5.2	4.8	6.3
6	80	175.5	3.8	2.1	5.4	3.1	11.4	6.5	13.1	7.5
7	80	400.5	15.5	3.9	20.4	5.1	22.6	5.6	34.1	8.5

Reproducibility (between laboratory sites) of the QUANTA Flash HMGCR assay was evaluated on 8 serum samples containing various concentrations of anti-HMGCR antibodies in accordance with CLSI EP5-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline. The samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per site. Between sites reproducibility was calculated and summarized in the table below.

			Within-Run		**Between-Day**		**Within-Site**		**Between-Site**		**Within-Laboratory**	
Sample ID	**N**	**Mean (CU)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**
Sample 1	75	16.1	0.6	3.4%	0.7	4.4%	0.9	5.6%	0.8	4.9%	1.2	7.5%
Sample 2	75	134.2	4.1	3.0%	7.7	5.7%	8.7	6.5%	0.4	0.3%	8.7	6.5%
Sample 3	75	123.9	3.6	2.9%	6.2	5.0%	7.2	5.8%	4.7	3.8%	8.6	6.9%
Sample 4	75	344.2	22.0	6.4%	18.3	5.3%	28.6	8.3%	14.8	4.3%	32.2	9.4%
Sample 5	75	22.8	0.8	3.4%	1.1	4.6%	1.3	5.7%	0.0	0.0%	1.3	5.7%
Sample 6	75	23.9	0.5	2.2%	1.0	4.2%	1.1	4.8%	0.5	1.9%	1.2	5.1%
Sample 7	75	10.6	0.3	3.0%	0.7	6.5%	0.8	7.2%	0.5	5.1%	0.9	8.8%
Sample 8	75	55.6	1.8	3.2%	3.0	5.5%	3.5	6.3%	0.0	0.0%	3.5	6.3%

Analytical Measuring Range

The analytical measuring range (AMR) of the assay is 1.5 CU to 550.0 CU. The linearity of the AMR was evaluated by a study according to CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Four serum samples with various anti-HMGCR concentrations were serially diluted to obtain values that cover the AMR. All 4 specimens showed dilution linearity individually, and the combined data yielded the following results with linear regression:

Serum Samples	Test Range (CU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	610.2 to 61.0	0.97 (0.92 to 1.01)	3.9 (-3.5 to 11.3)	1.00	98.5%
2	138.0 to 13.8	0.98 (0.95 to 1.01)	-0.1 (-1.3 to 1.05)	1.00	97.7%
3	21.5 to 2.2	0.94 (0.91 to 0.98)	0.0 (-0.2 to 0.2)	1.00	94.0%
4	10.4 to 1.0	1.03 (0.99 to 1.06)	0.0 (-0.1 to 0.1)	1.00	103.2%
Combined	610.2 to 1.0	0.98 (0.96 to 0.99)	0.1 (0.0 to 0.2)	1.00	98.4%

The Limit of Detection (LoD) of the QUANTA Flash HMGCR assay is 0.2 CU. It was determined consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 240 determinations, with 60 measurements on blank samples and 60 measurements of low level samples per lot. The Limit of Blank (LoB) is 0.0 CU.

The Limit of Quantitation (LoQ) for the QUANTA Flash HMGCR assay is 1.4 CU, determined consistent with CLSI EP17-A2 guideline, based on total imprecision of four low level samples tested for 3 days in replicates of 5 on two different reagent lots, obtaining a total of 30 determinations per sample. Total imprecision CV% to be <20%. LoQ is below the lower limit of the analytical measuring range.

QUANTA Flash® HMGCR Calibrators

For *In Vitro* Diagnostic Use

REF **701331**

Intended Use

QUANTA Flash HMGCR Calibrators are intended for use with the QUANTA Flash HMGCR Reagents for the determination of IgG anti-HMGCR autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

Summary and Principles of the Procedure

The QUANTA Flash HMGCR chemiluminescent immunoassay (CIA) utilizes a predefined lot specific Master Curve that is stored in the reagent cartridge barcode. The QUANTA Flash HMGCR 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 HMGCR CIA. Calibrators are tested on multiple instruments with multiple lots of reagents prior to value assignment.

Reagents

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

Warnings

1. All human source material used in the preparation of calibrators 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 HMGCR Calibrators should be handled in the same manner as potentially infectious material.[4]
2. Use appropriate personal protective equipment while working with the reagents provided.
3. 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 HMGCR Calibrators are for use with the QUANTA Flash HMGCR assay.
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 kept uncapped, onboard the instrument, 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 kept uncapped, onboard the instrument.

Procedure

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. 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 them 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 duplicate. 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 HMGCR Controls (sold separately – part number 701332) be run after a reagent cartridge lot is calibrated.

Traceability

No international standard serum for anti-HMGCR antibodies is available that allows for the standardization of anti-HMGCR antibody assays.

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 8 hours can result in improper calibration of the assay, which in turn could give erroneous results.

QUANTA Flash® HMGCR Controls

For *In Vitro* Diagnostic Use

REF **701332**

Intended Use

QUANTA Flash HMGCR Controls are intended for use with the QUANTA Flash HMGCR Reagents for quality control in the determination of IgG anti-HMGCR autoantibodies in human serum.

Summary and Principles of the Procedure

The QUANTA Flash HMGCR Controls are made up of a Negative Control and a Positive Control. Each contains a different amount of anti-HMGCR antibodies. The Negative Control and Positive Control are used to monitor the analytical performance of the QUANTA Flash HMGCR chemiluminescent immunoassay.

Reagents

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

Warnings

1. 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 HMGCR Controls should be handled in the same manner as potentially infectious material.[4]
2. Use appropriate personal protective equipment while working with the reagents provided.
3. 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 HMGCR Controls are for use with the QUANTA Flash HMGCR 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 an average time of 10 minutes onboard the instrument per use, for a total of 2 ½ hours.

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 an average 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. If the controls are left uncapped, onboard, for a total time greater than 2 ½ hours, they should be discarded.
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 HMGCR Assay:

1. Prior to using QUANTA Flash HMGCR Controls for the first time on the instrument, enter the name, lot, expiration, value (or dose), and target standard deviation (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 HMGCR 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 HMGCR 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 HMGCR assay in the column on the left. Then highlight the appropriate control material on the right (either "HMGCRN" for the Negative Control or "HMGCRP" 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 HMGCR assay from the list and click **Add**. Click **Save**. Perform this process for both controls.

It is recommended that the QUANTA Flash HMGCR Controls be used 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 instruments 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 standard serum for anti-HMGCR antibodies is available that allows for the standardization of anti-HMGCR antibody assays.

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. If the controls are left uncapped, onboard, for any longer period of time, they should be discarded.

References

1. Bottai M, Tjarnlund A, Santoni G, Werth VP, Pilkington C, de VM *et al*.: **EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: a methodology report.** *RMD Open* 2017, **3:** e000507.

2. Christopher-Stine L, Casciola-Rosen LA, Hong G, Chung T, Corse AM, Mammen AL: **A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy.** *Arthritis Rheum* 2010, **62:** 2757-2766.

3. Musset L, Allenbach Y, Benveniste O, Boyer O, Bossuyt X, Bentow C *et al*.: **Anti-HMGCR antibodies as a biomarker for immune-mediated necrotizing myopathies: A history of statins and experience from a large international multi-center study.** *Autoimmun Rev* 2016

4. **Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.** Centers for Disease Control/National Institute of Health, 2009.

Symbols Used

IVD	*In vitro* diagnostic medical device		Manufacturer
CE	European Conformity	EC REP	Authorized representative
Rx Only	Prescription Only per US FDA	Σ	Contains sufficient for < n > tests
	Consult instructions for use	CONTROL +	Positive Control
	Temperature limitation	CONTROL −	Negative Control
2	Do not reuse	CAL 1	Calibrator 1
	Biological risks	CAL 2	Calibrator 2
LOT	Batch code	PB	Recycle paper box
REF	Catalog number		This end up
	Use by		

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