## I. PRINCIPLE:

The Bio-Flash is an automated immunoassay analyzer designed for *in-vitro* diagnostic use. The assay analysis is based on chemiluminescent technology. In the solid phase of the reaction, assay buffer and magnetic particles coated with the assay specific antigen are combined. Patient sample is diluted and added to the mixture. After an incubation and wash step, isoluminol, a chemiluminescent tracer molecule, is added to bind to the anti-human antibodies. Following a second incubation and wash step, two triggers are added to generate the chemiluminescence and the light is measured. The amount of light emitted from the reaction is directly proportional to the amount of antibody present in the sample.

II. INSTRUMENTATION:

The Bio-Flash System comprises the following parts:

A. Analyzer
-Sample and reagent handling
- Testing and result measurement
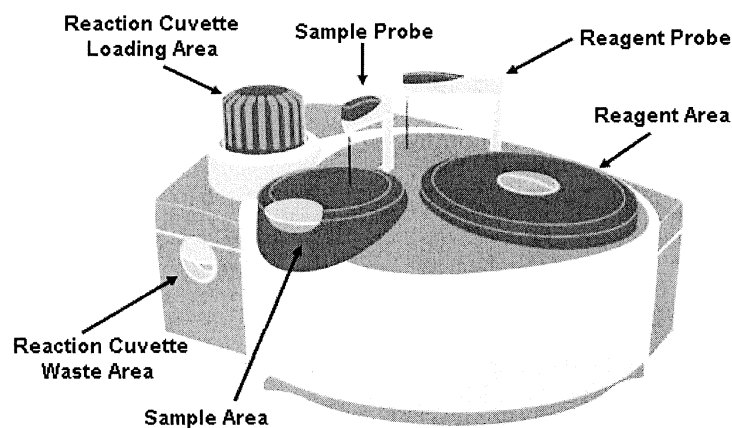
B. Computer
The computer provides:
- Data and instrument management and control and operation control
- Test materials management
- Fluid management
- Test tracking
- Communication with the LIS
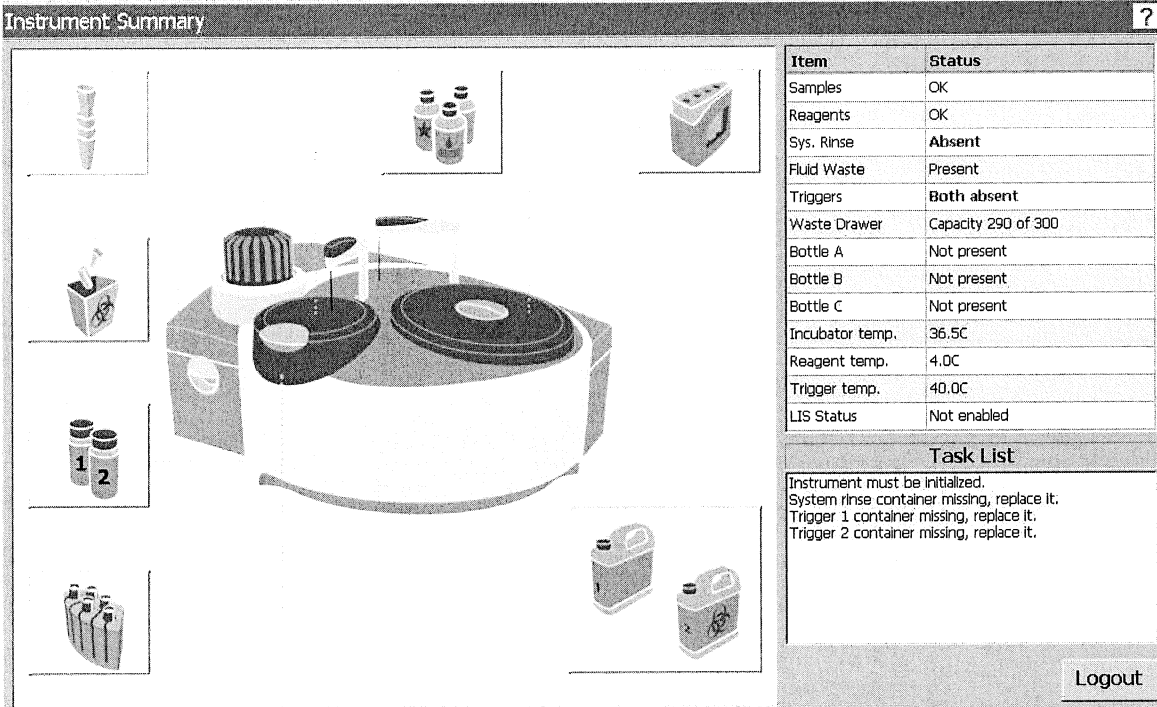- Touch screen and mouse capabilities to the operator

The Analyzer Function Units are the reaction cuvette loading area, reaction cuvette waste area, sample module, and reagent:

A. The **reaction cuvette loading area** is used to load stacks of cuvettes in columns into the instrument. Each stack contains 14 cuvettes and holds up to 20 columns allowing for a maximum of 280 cuvettes. The cuvette loader transfers a column of cuvettes into the dispenser column and separates individual cuvettes when needed.

B. The **reaction waste area** holds used cuvettes and can be manually emptied throughout instrument use.

C. The **sample module** is where patient samples are added and pipetted. Up to 6 barcoded racks can be loaded onto the carousel. Each rack holds 5 samples. Rack adapters are available for short specimens. The sample area is locked when the instrument is running, but can be unlocked once samples are done being processed. A sample probe transfers samples to the reaction area.

D. The **reagent module** is composed of a refrigerated reagent carousel and reagent probe. A maximum of 20 reagent cartridges can be loaded onto the carousel. Each cartridge contains four reagents, each specific to the individual assay. A mix head is present in the carousel to allow for intermittent mixings of the magnetic particles in each cartridge. A reaction probe transfers the reagents to the reaction area. If the instrument needs to be shut down for any reason, take the reagents off as the reagent carousel is kept at 1.5-6°C.

D. **Wet Section Unit** consists of the triggers, system rinse and waste. There are two triggers that are loaded on board necessary for the emission of light. Each is barcoded and kept in the enclosed cuvette waste area. System rinse and waste are both 5 L bottles that connect to the instrument. The system rinse is used in the magnetic wash state to purify the patient sample.

III. INSTRUMENT SUMMARY OVERVIEW:

The Instrument Summary screen provides comprehensive information on the state of the system and all the features needed to operate the Bio-Flash.

The inventory navigation buttons provide short cuts to the inventory screen. The inventory panel displays all present and/or missing materials and the system temperatures. The task list displays the status of Trigger, MagWash and Probe priming as well as the condition of cuvettes, reagent, triggers, diluents, rinse and waste sensors.

The **MENU BAR** options include:

1. BioFlash: opens the Instrument Summary screen for an overview of the instrument status.
2. Worklist: opens the worklist screen to create, view, print, delete or edit test items.
3. Results: opens the Result Database to view or print results listed in the database, archive results or search for results.
4. Pull-Down Menu options: allows user to view QC, Calibration, Maintenance and Configure options .

5. Start: starts the system based on a built worklist. Must be colored green to start the instrument.
6. Pause: pauses a test in progress. Turns yellow when instrument is in process.
7. Log: opens the instrument log. Flashing symbols alert user of a system problem.
8. Sample Inventory: opens Inventory-Sample Screen. Flashing symbols alert user of samples to be added.
9. Reagent Inventory: opens Inventory- Reagent Screen. Flashing symbols alert user of reagents required, missing, low or expired.
10. Bulks Inventory: opens Inventory- Bulks Screen. Flashing symbols alert user of information regarding triggers, system rinse and waste.

When an "i" or "!" is flashing on one of the icons, the instrument requires the operator's attention

The **STATUS DISPLAY** is at the bottom of the Info dialog and shows the current status of the system and the sample and reagent modules.

| | Sample cover unlocked | | Reagent cover unlocked | No work in progress System Status: Powered | Time remaining: --:-- User: |

IV. MATERIALS:
Refer to each assay procedure for reagents specific to each test. The materials listed below are universal among all assays.

1. System Rinse
   Intended Use
   Rinse used for the magwash portion of the chemiluminscent reaction.
   Composition
   A solution of potassium phosphate, potassium chloride, surfactant and <0.1% sodium azide.
   Preparation of Reagent
   The System Rinse is ready-for-use as supplied and requires no additional preparation
   Storage and Stability
   Store at 15 to 25 °C: The expiry date is given on the label
   Stability once opened: 3 ½ months

2. Triggers
   Intended Use
   Chemicals that trigger the chemiluminscent reaction
   Composition
   -urea peroxide (oxidizer)

-an iron containing porphyrin in 1 N sodium hydroxide (catalyst)
Preparation of Reagent
The Triggers are ready-for-use as supplied and requires no additional preparation
Storage and Stability
Store at 15 to 25 °C: The expiry date is given on the label
<u>**Stability once opened: 6 months onboard BioFlash**</u>

3. BioFlash Cuvettes
   Intended Use
   Used for the chemiluminescent reaction to take place. Stacks of cuvettes in columns are loaded into the carousel. The carousel holds up to 280 cuvettes: 20 columns, each containing 14 cuvettes/column. The dust cover stays on top of the carousel to prevent particulate matter from contaminating the cuvettes.

V.   ASSAY REAGENT LOT NUMBER INPUT:
Information regarding the reagent lot is stored on the reagent cartridge barcode. When the reagent is loaded onto the instrument and the barcode is scanned, the reagent details are uploaded into the system software. If a new lot is loaded on, the software will ask the operator to calibrate.

VI.   CALIBRATION LOT NUMBER AND ASSIGNED VALUES INPUT:
Information regarding the calibration curve is stored on the barcode of the calibration tube. When the barcode of the calibrator is scanned, calibrator details are uploaded into the system software.

VII.   CONTROL LOT NUMBER AND ASSIGNED VALUES INPUT:
When a new lot of control must be entered, use the worksheet provided inside the box of quality controls. Select QC from the drop down menu icon.
-Select "New QC Material" from the bottom of the screen. Enter the following information for each level:
- Fill in the name from the first column on the QC sheet
- Enter in the lot number from the sheet
- The barcode ID should automatically fill in as the name and lot number are entered
- Enter the expiration date from the QC sheet
-Change the status of the QC to "Active" from the menu
- Select "add assay" to select which assay the qc is testing
-Check off the following QC rules:
- 1(NS); enter 3
- 2(2S)
- N(x); enter 10
- Select Save when completed with each level of QC

VIII. OPERATION:

1. Verify Instrument/Software is ON:
- The BioFlash Instrument and Software should **always** be in the "on" mode.
    - If the BioFlash system was powered down for any reason, turn on the computer and the instrument. Select the BioFlash 2.2.1 Icon from the Desktop.
    - Log-in using "Manager" for username and "password" for password.

2. Perform Daily Maintenance
- Refer to the Daily Maintenance section of the procedure
- The instrument should be in ready mode and an initialization and prime should have occurred. Both are programmed to auto-prime and initialize every morning at 7 a.m.

3. Verify instrument supplies and load
- Review the Inventory Panel on the Instrument Summary screen for information, warnings or errors in relation to materials on the instrument. The Task List will also instruct the operator what needs attention.
- Load appropriate materials/reagents required for the instrument
    - **Reagents**: Refer to each assay procedure on appropriate reagent preparation.
        - To load reagent click the button on the toolbar that says "Reagent Cover" and open the cover when the system allows. Slip the front feet of the cartridge into the wheel. Snap the reagent cartridge into reagent carousel, making sure the back of the cartridge is firmly seated on the wheel. Ensure the magnetic bead vial can rotate freely once the cartridge is loaded. A mixhead in the wheel periodically mixes this portion of the cartridge during the shift. Close the reagent cover. A barcode reader inside the wheel automatically scans the wheel once the cover is closed.
        - To unload a reagent, unlock the reagent cover using the buttons on the status bar on the computer screen. Remove the cartridge and replace the reagent cover.
        - The Reagents screen shows the operator the details of reagent cartridges on board and provides navigation to detailed information about the cartridges.

    - **Triggers**: Select "Triggers" from the Inventory screen and follow the system prompts. Open the cuvette waste drawer exposing the trigger bottles. If you are not emptying the cuvette waste, select "no" at the system prompt. Use the handheld scanner and scan the barcodes for the trigger bottles as per the software's instructions.

Close the cuvette waste drawer. The computer software keeps track of doses left and the expiration dates.

- o **Cuvettes**: Remove the dust cover from the cuvette carousel. Place a full column into the slots and replace the dust cover. To empty the waste, remove the cuvette waste drawer and say "yes" to the system prompt. The instrument keeps track of the amount of space left for used cuvettes. Cuvettes may be loaded at any time, even when the instrument is running.
- o **Rinse**: To load new rinse, click the System Rinse icon from the inventory screen. Follow the system prompts and fasten the bottle cap onto the new bottle.
- o **Waste**: To empty the waste, click the System Waste icon from the inventory screen. Follow the system prompts.

4. **Request calibration** – if necessary.
Calibration must occur when:
- a new lot of reagent cartridge is put into use
- a specific QC violation occurs, i.e. 2-2s
- a new lot of QC is put into use
- major maintenance is done on the instrument
Refer to each individual assay procedure for details on calibration

   a. To request calibration:
      i. Load the two assay appropriate calibration barcoded tubes onto the instrument. Let the sample rack bar code read the racks and select play when it turns green.

      ii. When the calibration is finished, access the drop down menu from the menu bar and select calibration. A log of all of the calibrations is kept here. To see individual curves, double-click on the assay of interest. A sample calibration is show below. The solid line shown is the working curve or the line generated by the instrument test. The dotted line displays the master curve or the line generated by the manufacturer. Each assay has a different reference curve with its own %CV. The curve will pass or fail depending on the run. More than one calibration curve is kept in the database. A status box at the bottom left corner of the screen keeps track of the calibrations. The calibration in use is labeled "current." Curves that are not acceptable will be labeled as "invalid." Upon calibration, each curve must be validated by the operator. The status will change from "not validated" to "current" once the operator selects "Validate Calibration."

Calibration Parameters

Bottom:	230.5458	Top:	525859.9799
Hill Slope:	1.2054	Log	2.6791

Enter RLU: [ ]   Dose:

Average RLU Change: 5.641%
All calibration point CVs pass.
chi^2: 0.00189776
Valid
Calibrated using trigger lot 1009.

□ Logarithmic graph

Run At	Status
9/28/2012	Not validated
8/17/2012	Current

	Cal Name	RLU	Fit Value	Expected	Edited	Reps	Max CV	CV	Pass/Fail
1	C-IgM_aCLCAL01-1940	6415.80	12.1 CU	12.1 CU	No	3	8.00	0.32	PASS
2	C-IgM_aCLCAL02-1940	121352.00	175.6 CU	175.4 CU	No	3	8.00	3.02	PASS

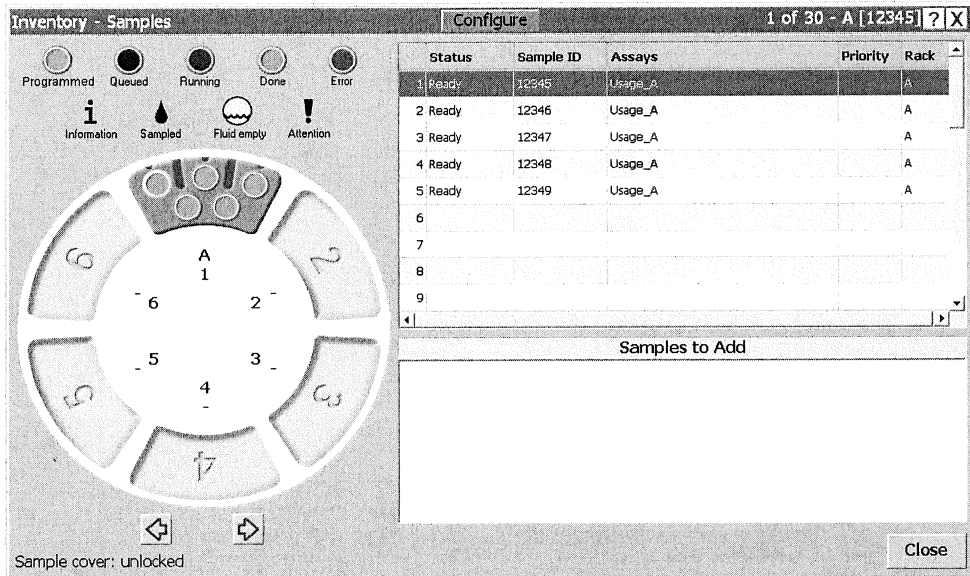| Validate Calibration | | Edit Point | Print | Close |

5. **Request controls:**
   - Load the assay appropriate barcoded tubes onto the instrument. Let the rack scan and wait for the play button to turn green. Press play and allow the assay to run.
   - To evaluate QC, press the drop down arrow and select QC from the list of options. Select the appropriate assay by double-clicking or by selecting the Levey-Jennings Icon.
   - For each shift, document control values using designated Excel spreadsheets found in L Drive → Immunology → QC CHARTS → BioFlash
   - For complete QC guidelines and explanations, refer to the Quality Control procedure Doc# IMM174.
     Refer to each individual assay procedure for details on quality control

6. **Loading Barcoded Samples on the Analyzer**
   - Sample racks are barcoded and each hold 5 patient samples. Load the patient samples with the barcode facing out through the opening in the rack. Select the "sample cover" icon to unlock the lid. If it's already unlocked, simply open the cover. Load the rack and close the cover.
   - The screen below automatically appears when the samples are loaded. The rack will scan and information regarding the samples are displayed in the box shown below.
   - Since the BioFlash is random access, as soon as the sample has been aliquotted, the rack outline turns green and the rack may be removed. The sample cover can be unocked and racks can be unloaded and loaded throughout the shift. A total of 30 patient samples can be loaded at one time (six racks total, each holding 5 samples), but more than that can be assayed at a time.

- A visual key is available to note the status of the onboard running samples. Once the samples are removed, the status is no longer available.

	Status	Sample ID	Assays	Priority	Rack
1	Ready	12345	Usage_A		A
2	Ready	12346	Usage_A		A
3	Ready	12347	Usage_A		A
4	Ready	12348	Usage_A		A
5	Ready	12349	Usage_A		A
6					
7					
8					
9					

Programmed   Queued   Running   Done   Error

Information   Sampled   Fluid empty   Attention

Samples to Add

Sample cover: unlocked   Close

7. Loading Non-barcoded Samples & Short Samples on the Analyzer
- Select the "Worklist" icon from the top Menu Bar. Select the "Racks" tab. It brings you to the screen show below.

	Sample ID	Assays	Needs	Received
1	<empty>			
2	<empty>			
3	<empty>			
4	<empty>			
5	<empty>			

h_tTG_IgG   PR3
IgA_aCL   RNP
IgA_B2GP1   Sm_
IgG_aCL   SSB
IgG_B2GP1
IgM_aCL
IgM_B2GP1
Jo1
MPO

☐ STAT

○ Plasma
○ Calibrator
● Serum

Available Racks
1   2   3

E   I   M
F   J   N
G   K   O
H   L   P

Sample ID: TEST~21

Tube/Cup: Cal Tube ▾

☐ No barc | Tube
Cup
Cal Tube

Create

>>   <<

\ Work Pending \ Add/Remove Assays \Racks/

Delete   Demographics   Comment   Log   Close
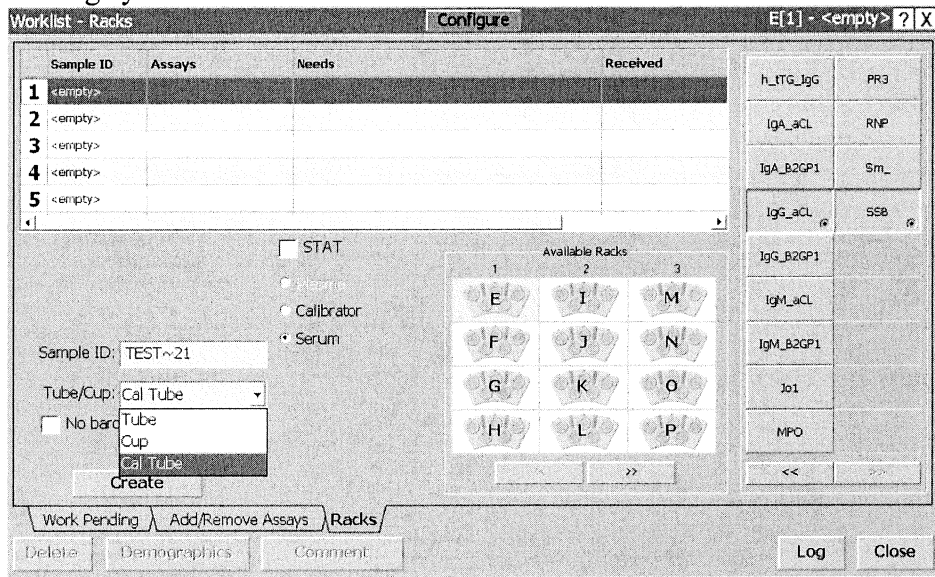
- Select the lettered rack you wish to use. Select the assay(s) from the tool bar on the right. Allocate the Sample ID you wish to use. It must be more than 2 characters. Keep the sample type as Serum. If the sample is STAT, check off the STAT box. Select the "No barcode" box. Indicate whether a tube or sample cup is being run. Select create.
  o For short samples, rack adapters and mirco vials are available for loading

- If more than one non-barcoded sample/short sample is to be loaded, select the next position and follow the same steps
- If more than one rack is to be loaded, select the next lettered rack and follow the same steps.
- Load the samples into the appropriate rack starting at position 1 (right most position).
- Load the rack onto the sample carousel, close the lid and press play.

IX.   REFERENCE CURVE VALIDATION/ACCEPTANCE CRITERIA:

ASSAY	% CV ACCEPTANCE
TTG IGA	8%
B2 IGG	8%
B2 IGM	10%

X.   RESULT APPROVAL:

1. Verify QC values are in control with use of designated Excel spreadsheet.
2. Click on each lab number that is ready for approval.
3. Click Approve

XI.   MAINTENANCE:

Daily:

1. From Instrument Summary screen, verify that items displayed in Inventory Panel are not in red. Any item in red must be corrected before processing any tests.
2. Observe Task List for any messages, warnings and/or errors. Address any that may be displayed.
3. Check Inventory – Bulks screen by clicking icon in menu bar or pressing F9. Available space for fluid waste container and cuvette waste drawer is shown. Click on Fluid Waste and/or Solid Waste to empty.
4. Also from Inventory – Bulks screen, if Triggers or System Rinse have low volume, click on their respective buttons to follow wizard and reload them.
5. Replenish cuvette loader by first removing dust cover from carousel top. Load cuvettes in full stacks of 14 where columns are empty. Carousel can be manually rotated to accomplish this. Do not load more than 14 per stack.

6. Clean reagent and sample probe with an alcohol wipe. To do this, Click ⬇ icon in menu bar and select **Maintenance > Clean Probes**. Follow the wizard; carefully wipe each probe straight down, from top to tip.

Monthly:

1. Perform System Cleaning by clicking ⬇ icon in menu bar and selecting **Maintenance > System Cleaning**. Have a System Cleaning Solution vial ready when cleaning wizard launches. When System Cleaning is finished, dispose of the cleaning vial and prime instrument. Select **Maintenance > Prime All**.
2. Complete monthly backup of Results Archive by first accessing Results Archive from Results screen. Select the month to be backed up to O Drive. Click Backup. Use O Drive → Immunology → BIO-FLASH → Results Archive (folder). Wait for Backup to finish and then close Results Archive window.

XII.   Appendix:

207		BioFlash Instrument Manual
207-A		BioFlash Instrument Checklist
207-B		BioFlash Instrument Quiz
207-M1		BioFlash Instrument Daily Maintenance
207-M2		BioFlash Instrument Weekly/Monthly Maintenance
207-Q		BioFlash Instrument QC Charts

XIII. References:
BioFlash® Operator's Manual. San Diego, CA: INOVA Diagnostics, Inc.; Revision 2.0, April 2011.

BioFlash Checklist
Doc# IMM 207-A
Instrument Checklist
Initial ☐   6 Months ☐

Instrument

YES/NO

1. Performs Daily Start-Up, Shut down of BioFlash.   _____

2. Perform daily, weekly and monthly maintenance of BioFlash.   _____

3. Can call BioFlash pending list.   _____

4. Able to calibrate and repeat an unacceptable calibration.   _____

5. Able to process daily controls.

6. Able to process barcoded and non-barcoded samples   _____

7. Able to independently load and run BioFlash without supervision   _____

8. Able to enter new lot of control or standard.   _____

9. Able to assess sample requirements for running of all assays.   _____

10. Able to interpret, approve and verify results appropriately   _____

11. Can perform basic troubleshooting of instrument or assay if   _____
    errors occur.

12. Understands all reagent stability requirements

13. Aware of inventory/par levels.
    a. Entry of all Lot #s in L Drive   _____
    b. Pretesting of all new Lot #s received.   _____

Quality Control

1. Able to ascertain QC validation by utilizing the   _____
   10X, 2-2S and 1-3S Westgard rules

2. Able to ascertain validation of standard curve.   _____

3. Proficient using SOFT Total QC   _____

Assay Checklist

Test	Read Assay Procedure Yes/No	Performed Assay-Dates Yes/No
B2 IgG	_____	_____
B2 IgM	_____	_____
TTGA	_____	_____
TTGG	_____	_____

Training Completed _____

Signature _____ Verified By _____
          Learning Technologist   Teaching Technologist

BIOFLASH – Maintenance log

Maintenance log _____
                              (Month/Year)

Weekly Maintenance procedure (Performed every Wednesday)

	WEEK 1	WEEK 2	WEEK 3	WEEK 4
1. Wipe reagent carousel with sports towel				
Remarks:				
Signature & Date:				

Monthly maintenance procedure

1. Run System Cleaning Solution	
2. Back up Results Archive to O Drive	
Remarks:	
Signature & Date:	

Supervisor review: _____   **Document # IMM 207-M2**

BIOFLASH – Maintenance log

Daily maintenance procedure

Maintenance log from _____ Month/Year _____

AM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1. View Instrument Summary and Task List for any errors/messages and address in needed																															
2. Check Inventory – Bulks screen to empty waste and/or replenish liquids																															
3. Replenish cuvette loader																															
4. Clean reagent and sample probe with alcohol wipe																															
Remarks:																															
Signature:																															

PM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
5. Check Inventory – Bulks screen to empty waste and/or replenish liquids																															
Remarks:																															
Signature:																															

Supervisor review: _____

Document # IMM 207-M1

Document Author
Katelyn Marreiros
Jimmy Debevoise
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Signature Approval for Annual Review
Name: BioFlash Instrument Manual
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Document #: IMM 207

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
TEODORICO LEE	LAB MANAGER	*(signature)*	5/20/13	NEW		5/17/2013
BRIAN SMITH	LAB DIRECTOR	*(signature)*	5/16/13	NEW		5/17/2013