YALE-NEW HAVEN HOSPITAL	TITLE: **AUTOBLOT 2000 & ViraCam Operation Procedures**		**DEPT OF LAB MEDICINE CLINICAL IMMUNOLOGY Policy and Procedure Manual**
			DOCUMENT # IMM 204
			Page 1 of 18
WRITTEN BY: Voula J. Kalmanidis	**EFFECTIVE DATE:** March 7, 2013	**REVISION:** NEW	**SUPERCEDES:** IMM168 Lyme Western Blot IgG & IgM MarDX

AUTOBLOT 2000

I. PRINCIPLE:

The MedTec AutoBlot 2000 fully automates the Western Blot assay. Following the manual addition of test samples, the AutoBlot incubates, washes, and performs subsequent reagent additions as defined by the operator during the programming phase. It permits easy setup with walk-away performance, sounding an alarm when the test is complete.

Assays are programmed into memory. Programs in the AutoBlot are made up of a series of individual steps, or tasks including incubation times, reagent volumes, rocking speeds, and cycles. The AutoBlot can save up to ten programs in memory. Once an assay is saved, simply recall it after *Ready For a New Test* and begin. If modifications are needed, recall the assay in Edit Mode, make the changes and resave it with the updates.

II. MATERIALS:

MT01001 20-strip trays (25 trays per pack)
MT01008 1000 ml waste bottle with lid
MT01049 pump tubing kit
MT01056 extension tubing kit
MT01011 peristaltic reagent pump
MT01012 power supply
MT01013 power cord
MT01018 complete shipping box

III. OPERATION:

1. Introduction:

The AutoBlot has been designed to provide simple, intuitive screen layouts to help guide the operator through the programming and automatic operation functions. By accessing

various modes in the system (Edit Mode, Pump Calibration, Pump Purge, Automatic Operation), assays are easily designed and executed.

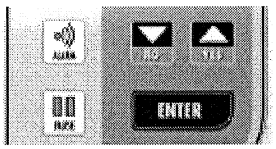
Ready For a New Test is the first prompt the operator receives when the AutoBlot is turned on. At this point, a previously programmed assay may be recalled and run. Or the operator may choose to enter Edit Mode to edit an existing assay or program a new assay.

Enter Edit Mode? is used for programming the steps of a new assay or editing the steps of a previously programmed assay.

Calibrate Pumps? is used to adjust the "on" time of each pump, allowing the operator to calibrate the volume dispensed from that pump. The AutoBlot comes factory-calibrated, but it is best to recheck the calibration annually or whenever pump tubing is replaced.

Purge Tubing? is the routine used to clear each pump line after an assay run.

There are five (5) keys on the keypad on the front of the unit:



Respond to prompts on the display screen by pushing one of these keys:

Press **ENTER** to make a selection or to tell the AutoBlot to proceed with a process.

Press **ALARM** to silence alarms that will activate at various stages in the program (alarms are used to get the attention of the operator - e.g., to get ready to prepare a solution or to indicate a process is ending).

Press **PAUSE** to temporarily halt the AutoBlot in order to prepare solutions, straighten a strip, etc.

The **YES** and **NO** arrow keys are used to select numbers and to accept or decline a process.

2. Overview - Running an assay:

When the AutoBlot is turned on, the display will prompt *Ready for a New Test.*
Press YES to enter Run Assay Mode.

Select the assay to be run from the list of assays displayed on the screen. Use the arrow keys to scroll through this list; select an assay by pressing ENTER.

Fill Bottles and Attach Tubing. All bottles should be washed thoroughly with CLRW before filling. Fill the wash buffer, CLRW, conjugate, and substrate bottles and place the correct tubing in each.

The pumps should be primed prior to running an assay. **If priming the pumps before the assay run, press YES at the *Prime Pumps?* prompt to enter Pump Prime Mode.** While in Pump Prime Mode the AutoBlot prompts the operator to prime each pump by pressing YES at the prompt for that pump. **Continue to press YES until fluid is dispensed into the drip tray.** Then press NO to continue to the next pump to be primed.

Indicate the number of strips to be run by using the arrow keys to increment through the numbers on the display. Press ENTER after selecting the correct number of strips.

Place the appropriate number of trays onto the tray platform.

Start the Test. Press YES at the *Start Assay?* prompt and the automatic operation begins. If the strip count needs to be reset, press NO at the *StartAssay?* prompt to return to the *Ready For a New Test* screen.

If an automatic soak cycle was programmed for the assay, soak (blocking) solution will now be dispensed into the trays. While the soak routine is in progress, prepare the other reagents and samples.

After the soak solution has been dispensed, the instrument pauses and the tray platform levels. Now add the strips to the trays. Gently tweeze the strips into the trays, being careful not to bend the strips (this may cause a strip to be picked up by the aspirate arm during the assay run). Strip number one (1) is located in the trough farthest from the aspirate arm in home position (looking at the front of the unit, strip one is farthest to the left). Any empty troughs should be located to the far left of the tray.

Any positive or negative controls should be placed in the troughs farthest from the aspirate arm drip tray. The negative control should be the furthest away (looking at the front of the unit, these controls will be to the far left). This will insure that if there is a problem (i.e. incorrect arm movement, loss of reagent, cross contamination) it will show up in the controls.

3. Automatic Operation

There are various features available during the automatic portion of the test. A description of these features follows:

Pause The PAUSE key may be pressed any time the tray platform is rocking (the tray rocks during incubation) in order to tweeze a strip, prime a pump, or skip to another step in the program.
After pressing PAUSE the test stops. The instrument remains in idle until a selection is made from the Pause Menu.

*1: **Return to Assay*** Restart the assay at the point of pause.
*2: **Prime Pumps?*** Enter the Pump Prime Routine.
*3: **Skip to Step?*** Restart the assay at a new step.
Note: PAUSE is not available while the instrument is dispensing or aspirating.

4. Alarms

The AutoBlot utilizes various alarms to get the operator's attention during an assay run. The operator should be familiar with these alarms, why they are on, and how to turn them off.

Reagent Addition Alarm
If an assay was programmed for manual addition of reagent, or if the reagent alarm was turned on when programming for automatic reagent addition, two (2) alarms will sound before the beginning of the incubation cycle. The first alarm comes on five (5) minutes before the end of the wash step and is an indication to prepare reagent. Press the ALARM key to turn off this alarm. A second alarm will come on as a reminder to prime the pumps.

Sample Addition Alarm
An alarm will come on with five (5) minutes remaining in the soak cycle. This allows time for the preparation of samples.
Press ALARM to turn off this alarm.
A second alarm will come on when the cycle is ready for samples. Press ALARM to turn off this alarm or YES if the samples are prepared.

NOTE: the platform will continue rocking until YES is pressed. After pressing YES, the trays sits idle until the samples are installed.

End of Assay Alarm The end-of-assay alarm comes on at the completion of an assay. Press ALARM to turn off this alarm and acknowledge the end of the assay. This alarm will automatically turn off after two (2) minutes.

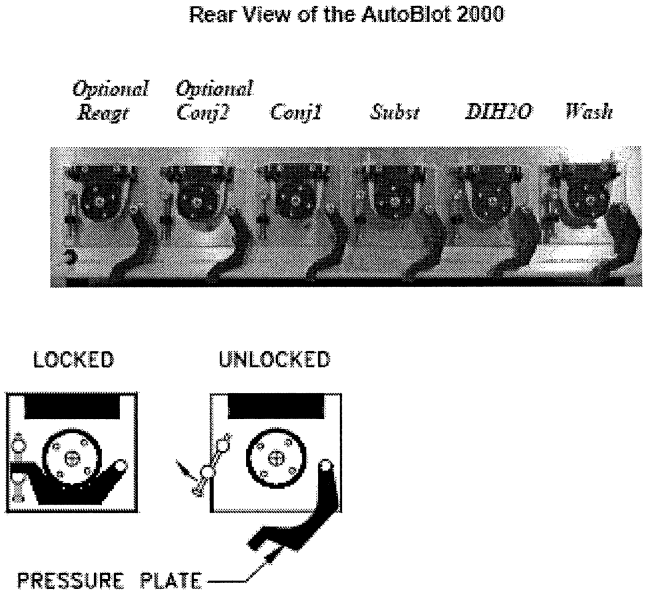
The AutoBlot now prompts *Purge Tubing?*
Press YES to purge the tubing or
Press NO to begin another assay.

As the automatic processing of the protocol begins, the various steps of the assay are displayed on the front of the instrument. Prompts come on at various points of the assay run instructing the operator to prepare samples, and to add reagents (if programmed for manual addition of reagents).

5. Running an Assay

Before beginning an actual test:

Prepare Wash Buffer	Mix the Wash Buffer and add it to the correct bottle.
Attach the Tubing	Attach the tubing to the bottles and snap the pump pressure pads in place.
Install the Tray(s)	Place the tray onto the platform for the test being run. Most assays are programmed to have soak (blocking) solution dispensed to the trays prior to adding the strips. Have the strips ready.
Lock Pump Pressure Pads	Snap the pump pressure pads in place.



Operating Instructions:

SCREEN DISPLAY	**ACTION**
Ready For A New Test ***Yes or No***	Press YES to begin a test. Press NO to enter Edit Mode.
Assay Name ***01***	Select the program to be run by using the arrow keys (< and >) to scroll through the available programs.
	Press ENTER to select a program and continue.
Pump Pads in Place? ***Press YES***	Press YES if the pump pressure pads are locked in place.
Prime Pumps?	Press YES to enter the PUMP Prime mode.

YES or NO	The pumps should be primed just prior to running an assay.
Prime Wash Line? ***Prime DI Pump?*** ***Prime Conjugate Pump?*** ***Prime Substrate Pump?*** ***Prime Stop Pump?***	Press YES at each prompt to prime that particular Pump. Be sure to press YES repeatedly for each Pump until fluid is dispensed into the drip tray. Then press NO to move on to the next pump to be primed. After all lines are primed, the display will return to *Prime Pumps?* Press NO to exit the Prime Pumps Routine.
Strip Count 20	Indicate the number of strips to be read by using the arrow keys to increment through the numbers on the display. Press ENTER after selecting the correct number of strips.
Load Tray ***Press ENTER***	Place the appropriate number of trays onto the tray platform. Press ENTER to continue.
Start Assay? ***Press YES***	Press YES at the *Start Assay?* Prompt and the automatic operation begins. If the strip count needs to be reset, press NO at the *StartAssay?* prompt to return to the *Ready For a New Test* screen.
Dispense Soak (Blocking) Solution	If an automatic soak cycle was programmed for the assay, soak (blocking) solution will now be dispensed into the trays.While the soak routine is in progress, prepare the other reagents and samples. After the soak solution has been dispensed, the instrument pauses and the tray platform levels. Now add the strips to the trays. Gently tweeze the strips into the trays, being careful not to bend the strips (this may cause a strip to be picked up by the aspirate arm during the assay run). Count the number of wells needed starting from the far right. Strip number one (1) is located in the trough closest to the aspirate arm in home position. Any empty troughs should be located to the far left of the tray.
Prepare Sampl Solution	During this time prepare samples; For Negative QC, Positive QC and Cutoff use 100 µL and for patients

	use 20 µL; Pipette samples left to right starting with Neg. Pos, Cutoff and patients
Sampl Prepared	PRESS YES (or silence alarm)
Sampl Installed?	YES OR NO; YES begins assay Pipet samples from left to right beginning with Negative QC, Positive QC and Cut-off; then pipet Specimens

AUTOBLOT 2000 will incubate, wash, add conjugate and substrate.

The entire assay takes approximately 90 minutes.

Alarm will sound at end of assay.

Remove Tray and lightly tap to remove excess CLRW.

Using an applicator stick, move strips up leaving approximately ½ inch from the end of the well. Allow strips to dry.

When strips are dry, reverse tray so that strips begin at the left hand side of the tray as this is where the ViraCam starts reading.

IV. MAINTENANCE:

DAILY:

1. Purge Tubing Routine

The tubing for each pump should be purged at the completion of an assay run.

Entering Purge Tubing
The Purge Tubing Routine is accessible at the end of an assay or after the Pump Calibration Routine.
Use a 2% bleach solution (2 mL of generic brand bleach to 98 mL CLRW).

SCREEN DISPLAY	**ACTION**
Purge Tubing? ***YES or NO***	Press YES to enter the Purge Tubing Routine. Press NO to return to *Ready For A New Test*
Auto Flush Lines? ***YES or NO***	Press YES to automatically flush the pump lines. Press NO to flush the pump lines manually. If manual flushing is selected, the AutoBlot will guide

	the operator through the process with a series of screen displays. Press ENTER to continue ***Manual Mode** To clean the tubing manually, select NO at *Auto Flush Lines?* In manual flushing, the system will pump 10 mL through each tube***
For Auto Flush -	. Place the tubing in an appropriate cleaning solution.
Place Tubes in Cleaner Press ENTER	Press ENTER to start the pumps which will run cleaning solution through the tubes. The tubes will soak in the cleaning solution for five (5) minutes. An alarm will sound at the end of the soak time.
Put Tubes in DI Press ENTER	Place the tubing in CLRW. Press ENTER to start the pumps which will run CLRW through the tubes. The tubes will soak in the CLRW for five (5) minutes. An alarm will sound at the end of the soak time.
Remove Tubes From DI Press ENTER	Remove the tubing from the CLRW and place the tubing on paper towels to dry. Press ENTER to flush the CLRW from the tubes.
Release Pressure Pad Press ENTER	Unlock the pump pressure pads. Press ENTER to continue. Display prompts *Purge Tubing?* again. Press NO to exit this mode and return to *Ready For a NewTest*

Turn off AutoBlot 2000

2. Bottles

Wash out all bottles after running an assay to ensure a clean solution for the next assay. Use CLRW and a 2% Bleach Solution.

3. Instrument Cleanup

At the end of each day, Wipe down the instrument with a damp paper towel.

4. Aspirate and Dispense Tips

Using isopropyl alcohol, wipe off the surfaces of the aspirate arm and dispense arm that come in contact with each other. These tips must be kept clean in order to prevent clogs and to ensure that fluids are properly aspirated and dispensed. In order to prevent possible contamination, **DO NOT** use the same swab on the dispense tips that it used on the aspirate tips.

WEEKLY:

1. Aspiration Timing

Aspiration timing should be verified weekly during operation. During the aspirate/dispense routines, press PAUSE and lift the cover when the aspirate arm is lifting up, preparing to index to a new position. Check the trough that was just aspirated to make sure it is empty. Do this at several different trough locations throughout the assay.

ANNUAL:

1. Tube Replacement

Tube replacement kits are available from MedTec. The tubing in these kits is cut to the proper length and marked for proper alignment.

NOTE: Proper tube lengths and installation are critical for proper performance of the arm assembly.

The pump tubing is the short length of tubing that wraps around the pump. The pump tubing manufacturer gives a tubing life of approximately 1000 hours. The pump tubing should be changed annually

2. Pump Calibration

It is best to prime the pumps before calibrating them. The AutoBlot 2000 will prompt the operator to prime the pumps before the calibration routine begins. It then leads the operator through the calibration exercise for each pump.

NOTE: Before the pumps are calibrated, make sure the pressure pads have been locked for at least one (1) hour in order to approximate real-life operating conditions. It is also best to prime the pumps before calibrating.

For each pump to be calibrated, remove the pump line (including the plastic tip) from the dispense arm and place it in a graduated cylinder. Remove only one line at a time so as not to confuse the proper line configuration. The pump will dispense 40 mL (twenty 2mL dispenses). Check the actual volume dispensed into the cylinder and enter this number into the AutoBlot. If it was not exactly 40 mL the instrument will make corrections and try again to dispense 40 mL for that pump. It will continue to do this until the operator verifies that 40mL has actually been dispensed. The calibration for that pump is then complete, and the program advances to the next pump for calibration, until all pumps have been checked.

The Pump Calibration Routine is used to adjust the "on" time for each pump in order to calibrate the volume dispensed from that pump. This routine is accessed by entering NO at the *Enter Edit Mode?* prompt.

Before the pumps are calibrated, make sure the pressure pads have been locked in place for at least one hour in order to approximate actual operating conditions.

SCREEN DISPLAY	ACTION
Calibrate Pumps? ***YES or NO***	Press YES to enter Pump Calibration. Press NO to bypass this routine and go to Pump Purge.
Pump Pads in Place? ***Press YES***	Press YES to acknowledge pump pads are locked in place.
Exercise Pump Tubes? ***YES or NO***	The tubing that comes with the unit has already been broken-in (exercised). However, if new tubing is installed, it should be exercised before the next assay run. This routine will exercise each tube for approximately 20 minutes. Press YES to exercise the new tubing. Press NO to bypass this routine and go to Pump Calibration.
if YES: ***Exercise All Pumps?*** ***YES or NO***	Press YES to exercise all pump tubing. Press NO to exercise individual pump tubing.
if NO: ***Exercise Wash Pump*** ***YES or NO***	Press YES to exercise the Wash Pump. Press NO to decline this pump and exercise the next pump.
if NO to Exercise Pump Tubes?	
Calibrate Now? ***YES or NO***	Press YES to enter the Pump Calibration Routine.

Press NO to access the Purge Tubing Routine.

if YES:

Prime Wash Pump? YES or NO

Before the Pump Calibration Routine begins, the operator has the option of first priming the pumps.

Press YES to prime the pumps before calibrating.
Press NO to skip pump prime and go directly to pump calibration.

Dispense 40 ml Wash? YES or NO

For each pump to be calibrated, remove the pump line (including the tip) from the dispense arm and place it in a graduated cylinder. Remove only one line at a time in order to maintain the proper line configuration.

Press YES and the Wash pump will dispense 40 mL water (twenty 2 mL dispenses).

Press NO to skip the Wash pump and go to the next pump.

Actual Volume Dispensed 40 ml

Check the graduated cylinder for the actual volume dispensed. Enter this number at the screen prompt. If the actual volume was 40 mL then the calibration for that pump is complete.

If the volume dispensed was not exactly 40 mL, the AutoBlot will make corrections and attempt once again to dispense 40 mL for that pump. It will continue to do this until the operator verifies that 40 mL has actually been dispensed. Then, the calibration for that pump is completed and the program moves on to the next pump for calibration, until all pumps have been checked.

ViraScan & ViraCam

I. Principle:

ViraScan® is an analysis software for the evaluation, assessment and documentation of ViraBlot® and ViraStripe® tests. The ViraCam® reading equipment is designed to directly read test strips from the reaction tray. Bitmap images (8 bit greyscale) are generated to be further analysed by the ViraScan® software.

II. Operation

1. Preparation of test strip reading

Please make sure that all strips are well dried and plane to prevent from mirroring effects or shadow production.

Open the drawer of the ViraCam® device. Place the black tray containing the developed test strips onto the drawer. Align the tray to the left edge and back stop. Well # 1 should be on the left side (close to the left edge of the drawer). The labeled side of the test strip is directed towards the front side of the drawer. The drawer must be closed carefully prior to the scan process.

Use a **cut off** strip for every test run of ViraBlot® assays.
ViraStripe® assays include a cut off control band on every antigen strip within the integrated control system.

Start ViraScan® by double-clicking on the **ViraScan** icon.

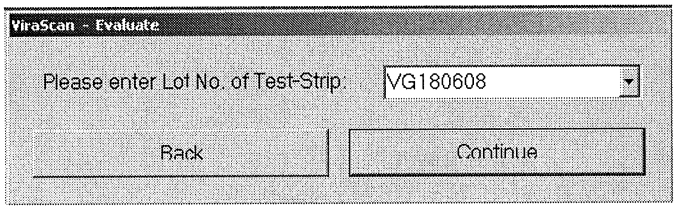
Click on the pull down menu "**process**". The options are "**scan**", "**prepare**", "**evaluate**", "**print**" and "**end**".

The option "**prepare**" allows to define a worksheet, prior to the evaluation, with patient data for each specific strip. The entered information should be saved under the lot number of the antigen strip, and is used for the automatic interpretation. In option, the worksheet can be printed. Alternatively, patient data for specific strips can be entered or changed during the process "**evaluate**".

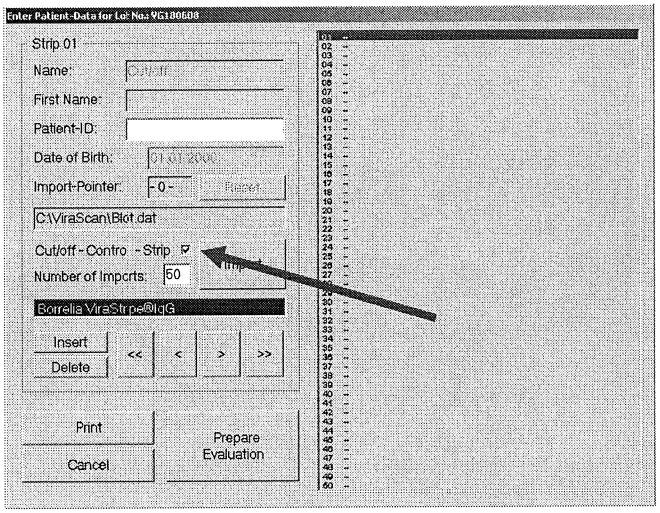
Click on the option "**prepare**" in the pull down menu "**process**" at the ViraScan® start window. Enter the appropriate antigen strip lot number and confirm with "**continue**".

INSURE VIRACAM INSTRUMENT IS ON

2. Prepare Worklist
    a. From the drop down menu, click on process
    b. Then click on Prepare
    c. Enter the File Name: LGMMDDYYYY or LMMMDDYYYY
    Ex. LG02062013
    (screen indicates Lot No. of Strip but this is used as run number)
    Always start with LG (IgG) or LM (IgM) for the run file name.



    d. Continue
    e. Cancel *.imp (Import from LIS)
    f. Enter or scan Patient ID (beginning with Negative and Positive QC)
    g. Indicate the position of the Cut-off by clicking in the Cut-off Control-Strip box

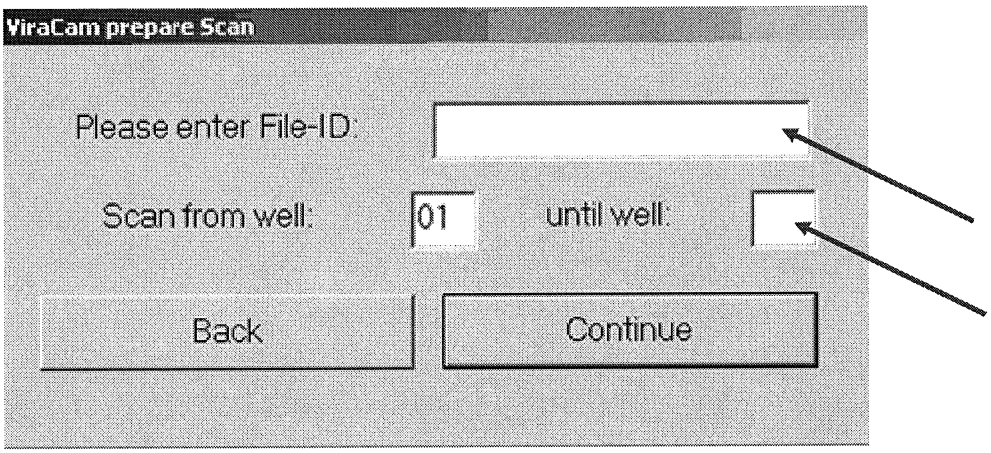


    h. Click on the > button
    i. Then click in the Patient ID field to continue entering/scanning Patient ID information
    j. To print a worklist
        i. click on Print

ii. select desired printer (HP LaserJet Professional P 1102w)
iii. Click on Print

k. Click on Prepare Evaluation

3. To Scan Strips
   a. From the drop down menu, click on process
   b. Click on Scan
   c. Enter **File Name (LGMMDDYYYY or LMMMDDYYYY)**
   d. Select: Well 1 – 29 (default is 1 to 50)
   e. Continue
   f. A DOS screen will appear indicating that the lamp is warming up (in German); When scanning is complete, the window will disappear.



4. Evaluate
   a. From the drop down menu, click on process
   b. Click on Evaluate
   c. Enter File ID (will default to last File Name entered)
   d. Click on Continue
   e. Enter Band Locator; *LG or LM* for IgG or IgM, respectively
   f. Enter location of Cut-off if not displayed, this is the actual well number in which the cut-off is located.
   g. Enter Lab Name and examiner (tech)
   h. Click on Continue to evaluate/review strips
   i. Use the >> button to advance to the next sample
   j. Advance one more time after final strip, the print button becomes available
   k. Click on Print
      i. Select desired printer (HP LaserJet Professional P 1102w)
      ii. Click on Preferences
      iii. Click on icon of a "document" to change orientation to landscape
      iv. Click OK
      v. Click Print

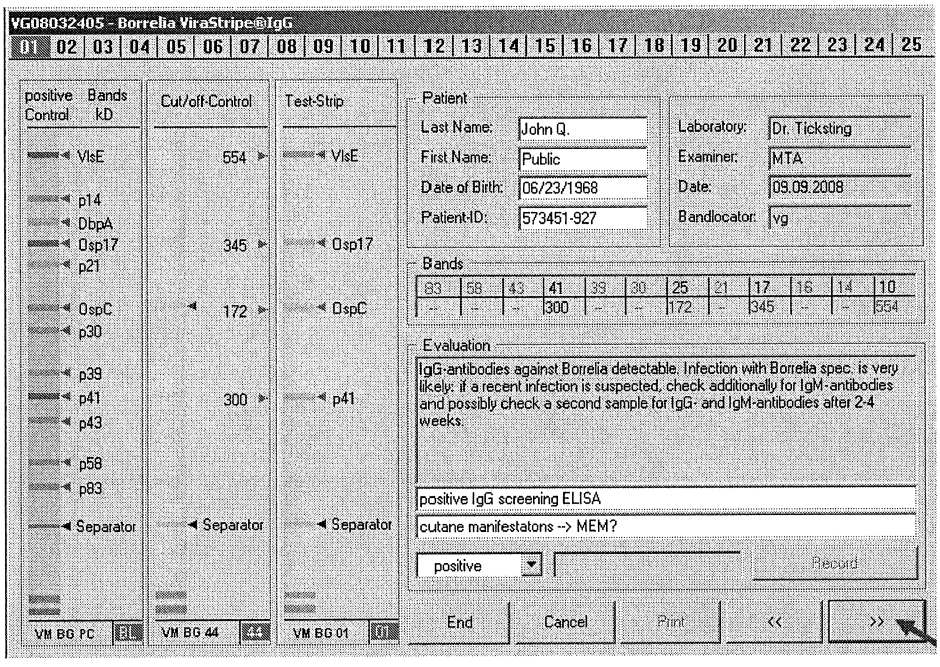
l. When both the IgG and IgM strips for a given run have been completed, the operator can utilize the record button to toggle back and forth between IgG and IgM results.
m. DO NOT USE the Evaluate function to review scans that have already been evaluated as clicking on Print or END will overwrite results. See # 5, Archive for this function.

5. Archive
    a. To Print Individual Reports or to review results for trays already scanned and evaluated
    b. Select Lot#
    c. Enter or pull down **File ID**
    d. Select and print out desired Print-out

6. Review / Evaluation of results

In the evaluation screen, the first strip on the left shows the positive control from the lot template indicating the bands of the Borrelia ViraStripe® IgG or IgM. The second strip in the middle shows the cut-off strip for this evaluation. The intensity of the cut-off band is set to the value 60, which is the level giving the highest concordance with visual reading. The third strip on the right shows the patient strip of the selected strip number.

The found bands are indicated on the right below the patient information. The intensity of these bands in relation to the cut-off is indicated below the band names.



The found bands and their intensity values are listed left and right to the

patient strip. Switch to the different patient results by clicking on the buttons ">>" or "<<" or by clicking directly on the corresponding strip number.

III. Appendix:

	Instrument Manual
IMM204-A	Instrument Checklist
IMM204-M1	Instrument Maintenance

Doc# IMM204-A
Instrument Checklist
Initial ☐ 6 Months ☐

Instrument

	YES/NO
Performs Daily Start-Up, Shut down of AutoBlot 2000.	_____
Perform daily, weekly and monthly Maintenance of AutoBlot 2000.	_____
Can call a pending list.	_____
Able to independently load and run without supervision	_____
Able to assess sample requirements for running of all assays.	_____
Can perform basic troubleshooting of instrument or assay if problem occurs.	_____
Understands all reagent stability requirements	_____
Understands and can utilize the ViraScan Software	_____
Able to prepare a worklist	_____
Able to scan strips	_____
Able to evaluate strips	_____
Can recognize if strips are not properly read/evaluated	_____
Able to utilize the ViraCam scanner	_____
Aware of inventory/par levels.	
Entry of all Lot #s in L Drive	_____
Pretesting of all new Lot #s received.	_____

Quality Control

Able to ascertain QC validation	_____

Assay Checklist

Test	Read Assay Procedure Yes/No	Performed Assay-Dates Yes/No
B. burgdorferi IgG	_____	_____
B. burgdorferi IgM	_____	_____

Training Completed _____

Signature _____ Verified By _____

Learning Technologist Teaching Technologist

AUTOBLOT 2000 Month: _____

ID # CAB0899-257

Daily	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
Purge Tubing Routine																															
Wash Bottles																															
Wipe down Instrument																															
Clean Aspirate & Dispense Tips w/alcohol swab																															
Date																															
Initials																															

Weekly	1	2	3	4	5
Aspiration Timing					
Date					
Initials					

Annual	1	2	3	4	5
Tube Replacement					
Incubation Timer Verification					
Pump Calibration					
Preventative Maintenance - Manufacturer					
Date					
Initials					

Supervisor's Review	1	2	3	4	5
Date					
Initials					

Document Author
Voula J. Kalmanidis
March 7, 2013

Signature Approval for Annual Review
Name: AUTOBLOT 2000 and ViraCam Operation

Document #: IMM204

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	Teodorico Lee	3/6/13	NEW		3/7/13
BRIAN SMITH	LAB DIRECTOR	BLH	3/6/13	NEW		3/7/13