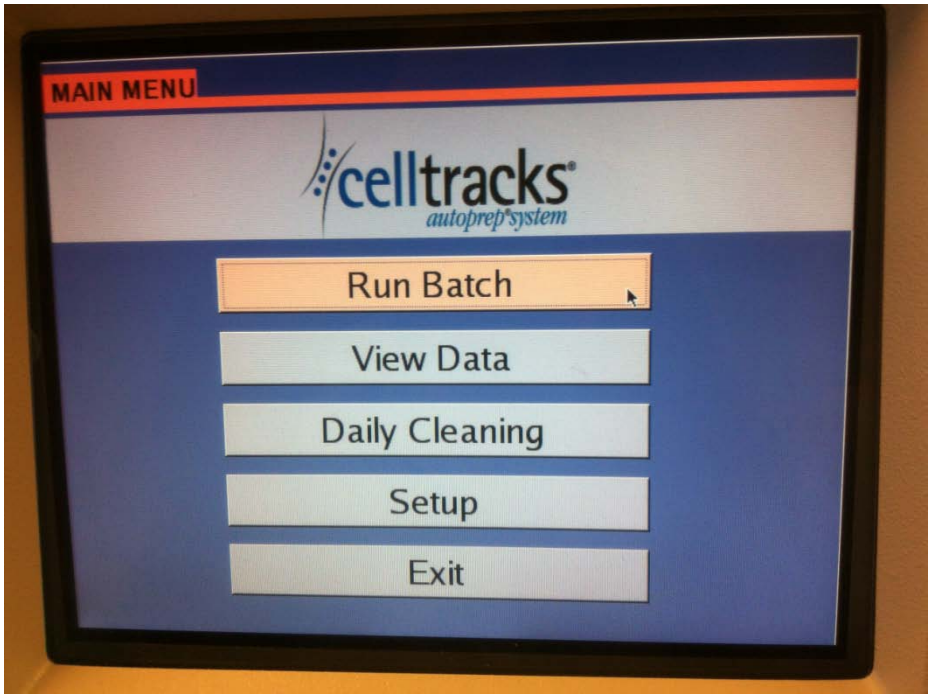
Standard CTC Enumeration from Patient Blood Samples using the CSS

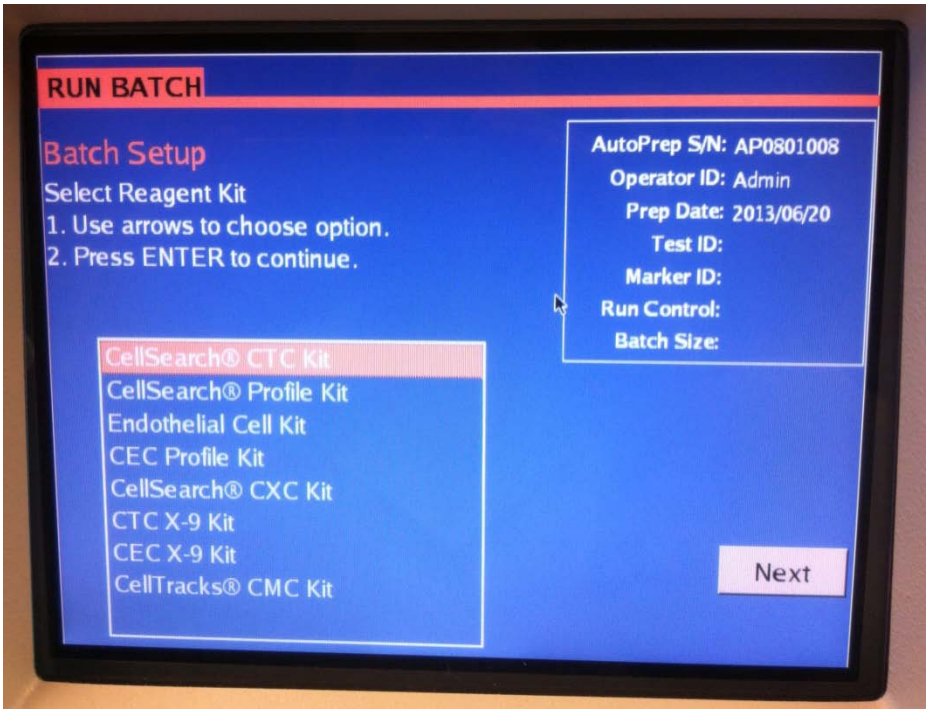
1. Human Blood Sample Collection and Preparation for Processing on the Preparation Instrument

1.5 Follow the on-screen instructions on the preparation instrument to load all patient samples onto the system for processing. Samples must be processed within 1 hour of preparation.

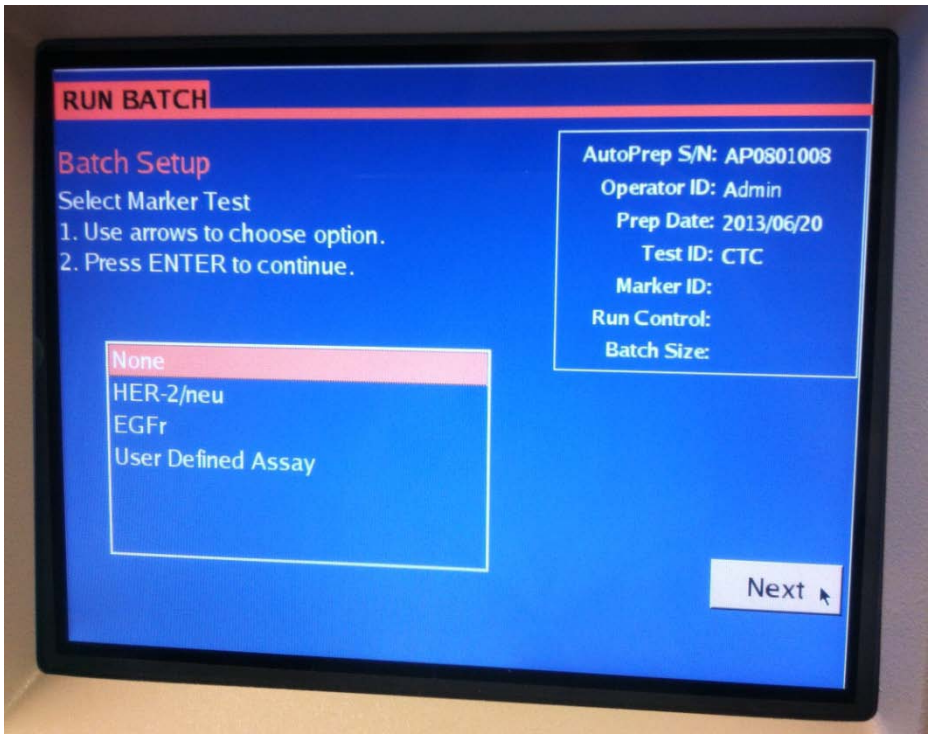
***** Images below also depict steps*** described in section 2.4 of the ***Standard CTC Enumeration from Patient Blood Samples using the CSS.***



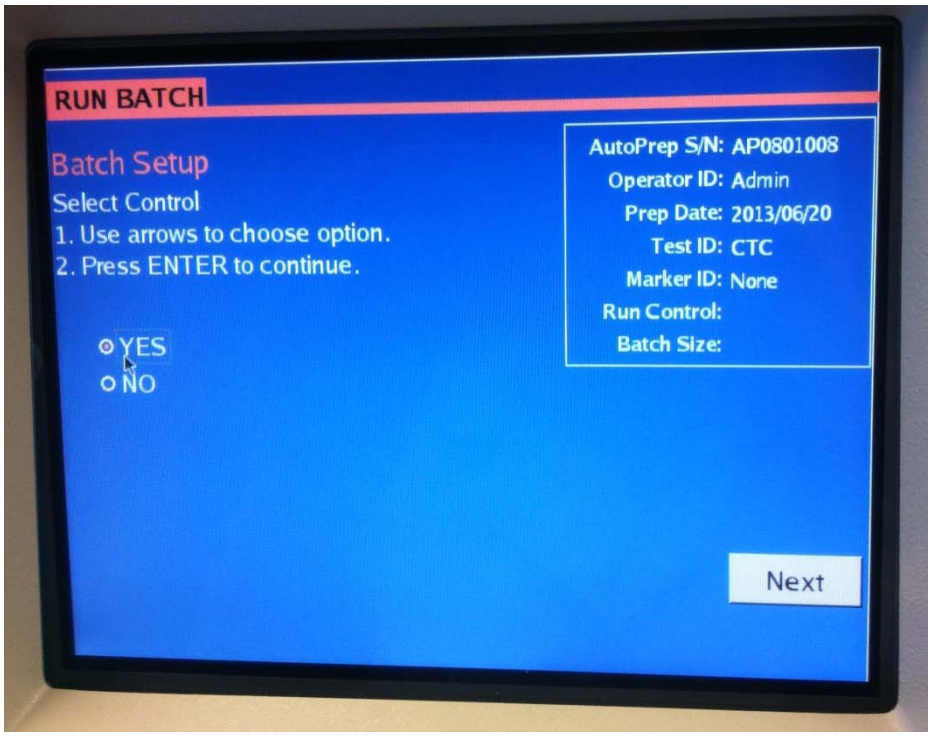
1.5.1 Select **Run Batch** from main menu.



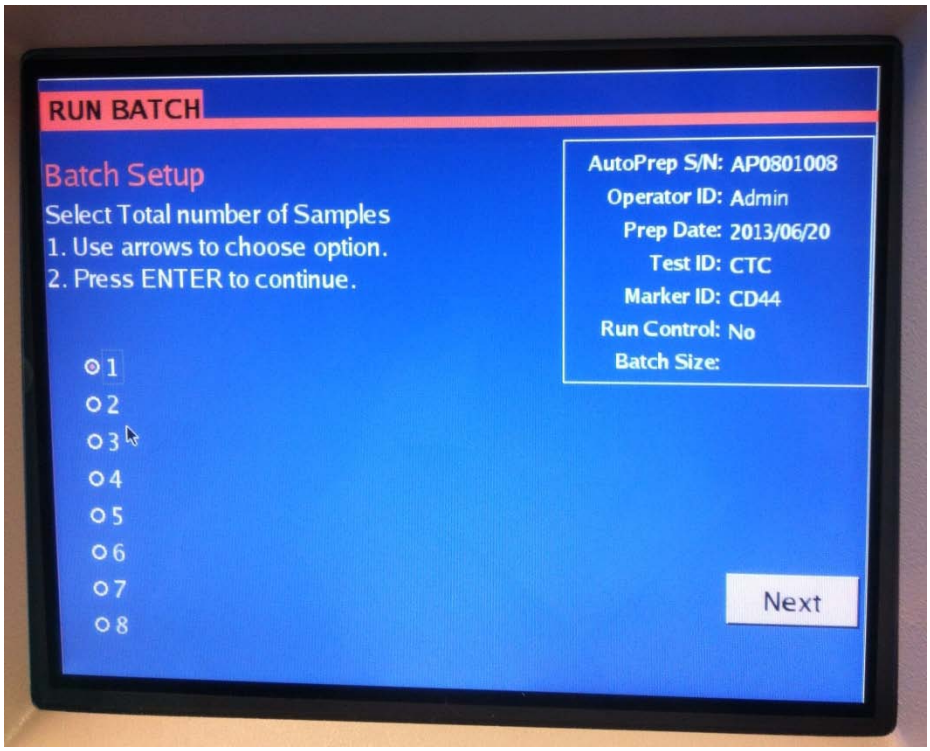
1.5.2 Select **CellSearch CTC Kit** and click **Next**.



1.5.3 Select **None** and click **Next**.

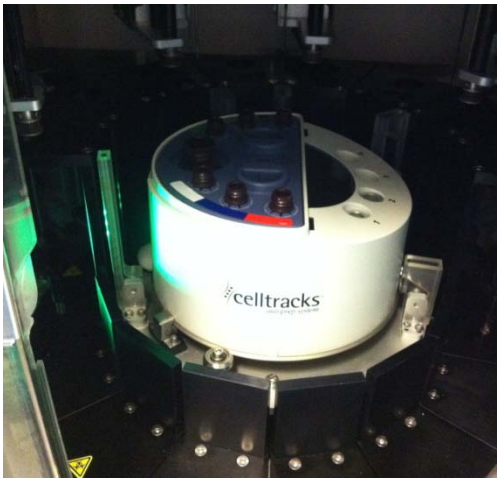


1.5.4 Select **Yes** (run a control) or **No** (do not run a control) as applicable and click **Next**.



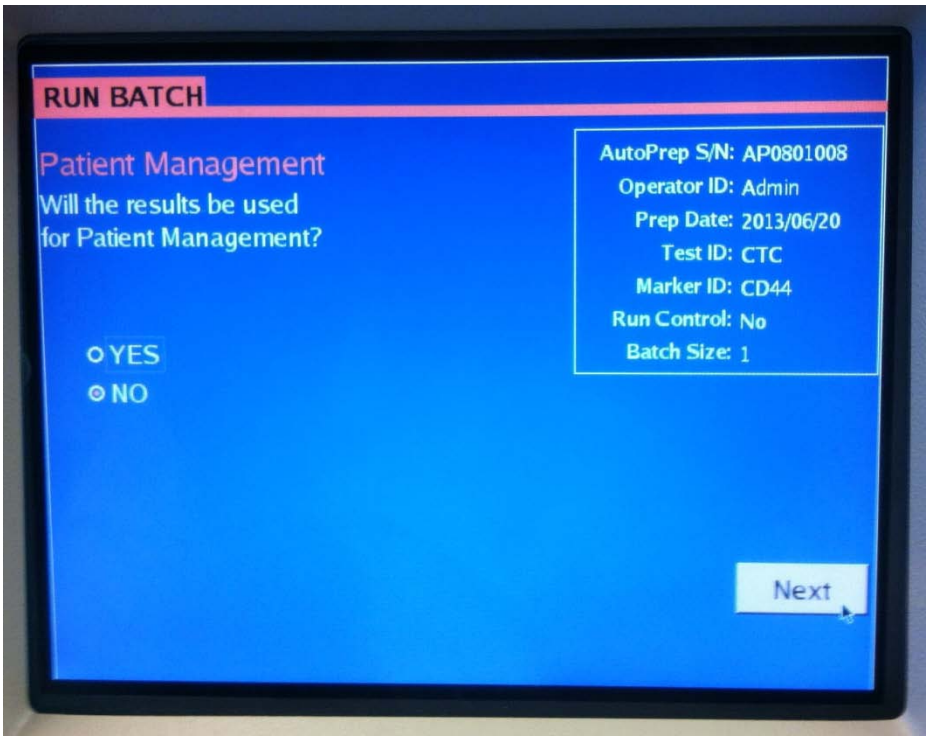
1.5.5 Select the number of samples that will be processed (including the control, if applicable) and click **Next**.

(A)



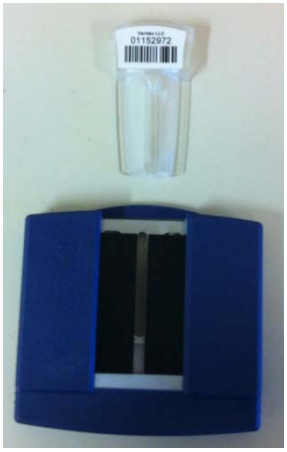
(B)

1.5.6 Load the CellSearch CTC kit onto the AutoPrep (A) and click **Next** (B).



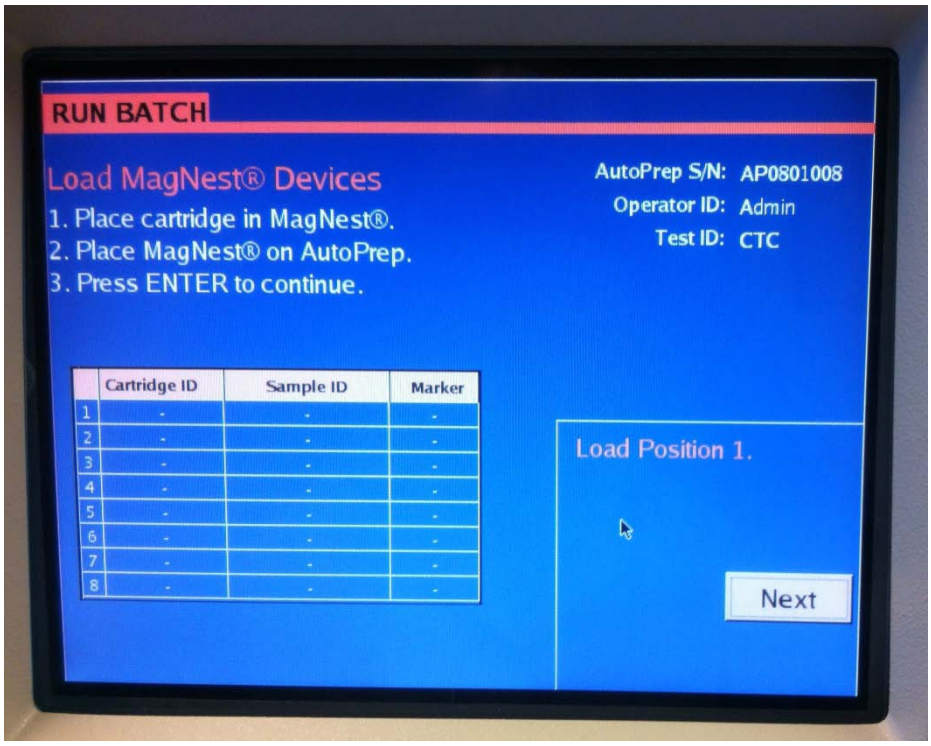
1.5.7 Select **Yes** (results will be used for patient management) or **No** (results will not be used for patient management) as applicable and click **Next**.

(A)



(B)

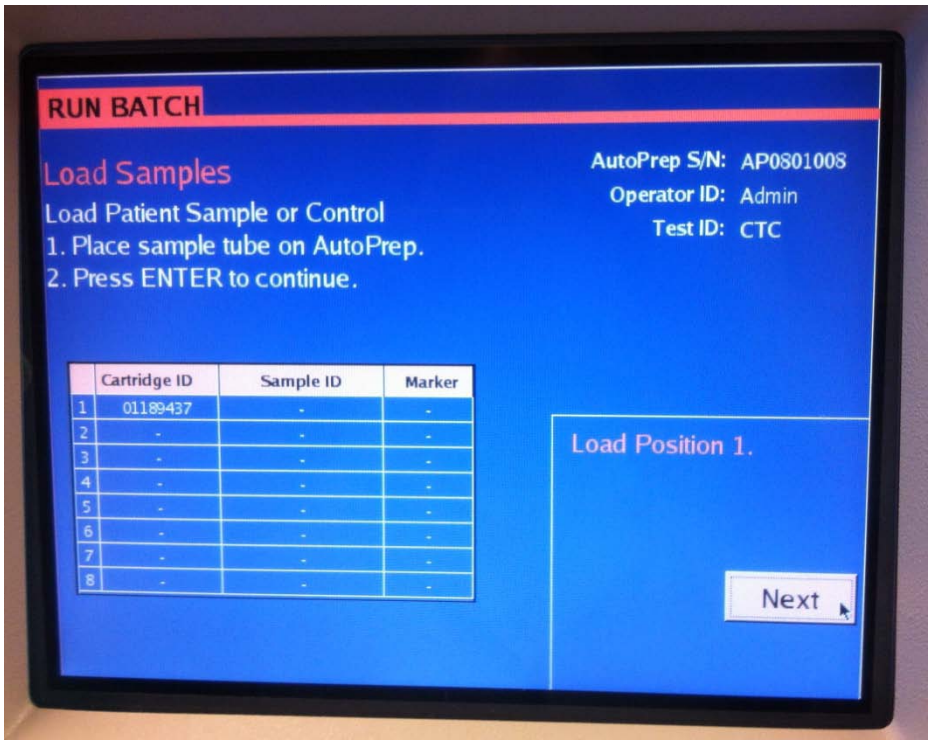
(C)



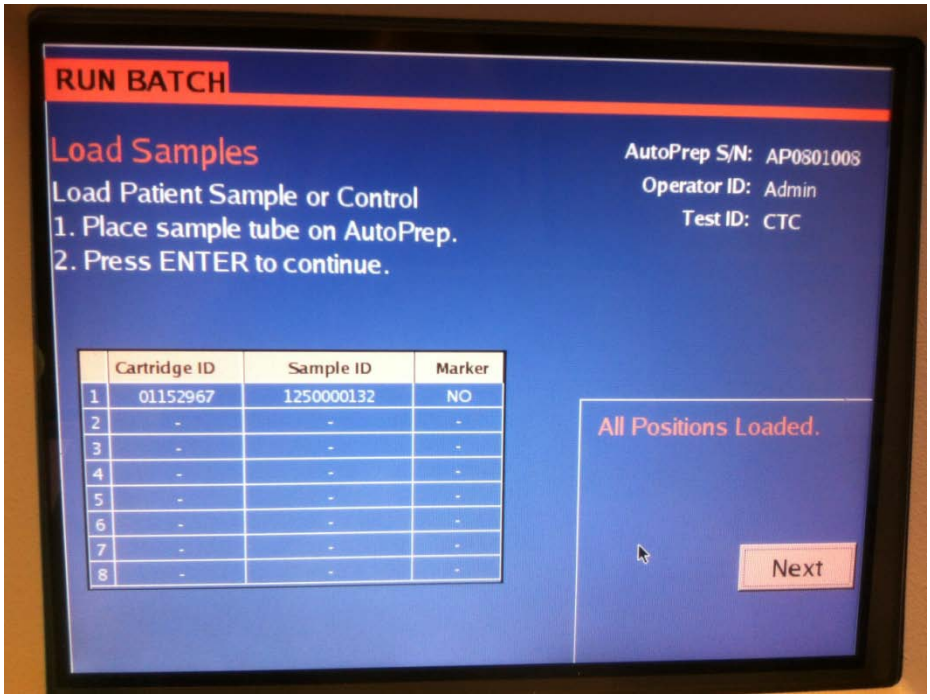
1.5.8 Place the MagNest cartridge in the MagNest (A), place the MagNest on the AutoPrep (B), and click **Next** (C).

(A)

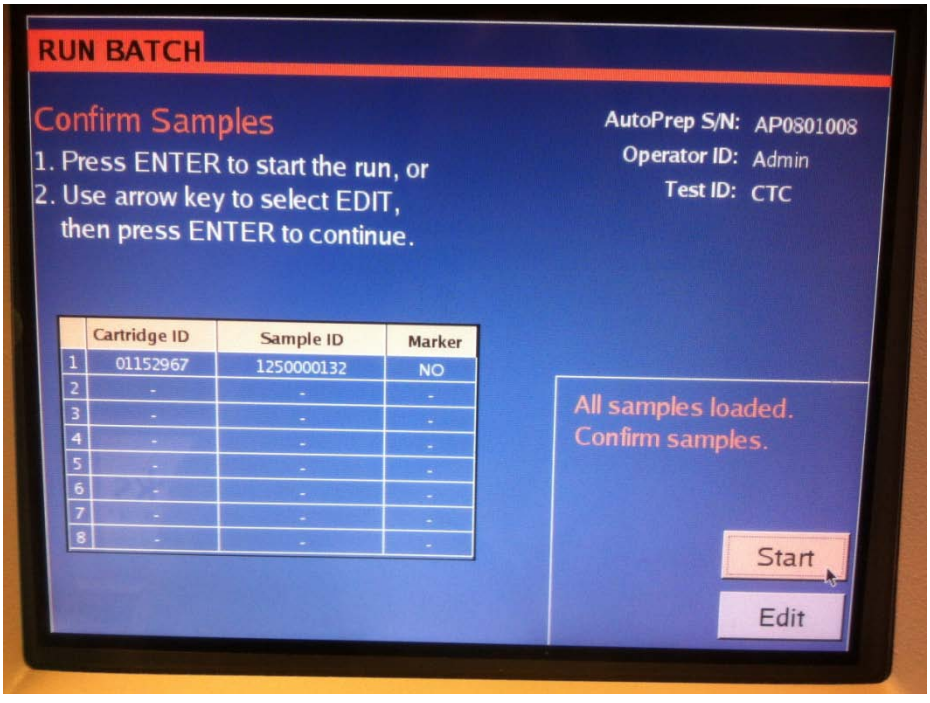
(B)



1.5.9 Load the patient sample or control onto the AutoPrep (A) and click **Next** (B).



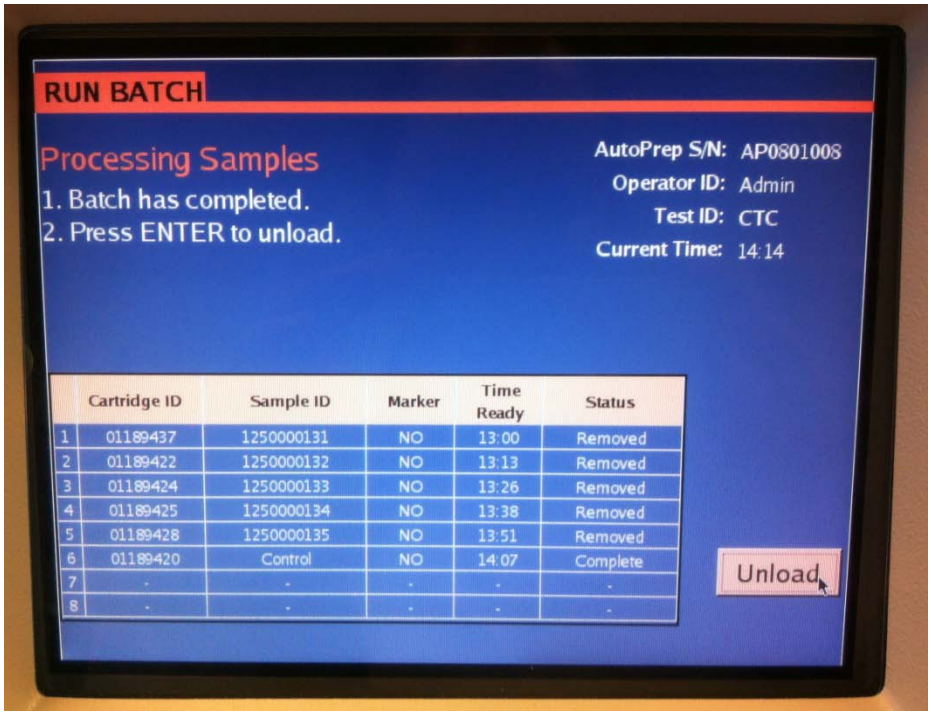
1.5.10 Confirm that all positions are loaded and click **Next**.



1.5.11 Click **Start** to begin sample processing.

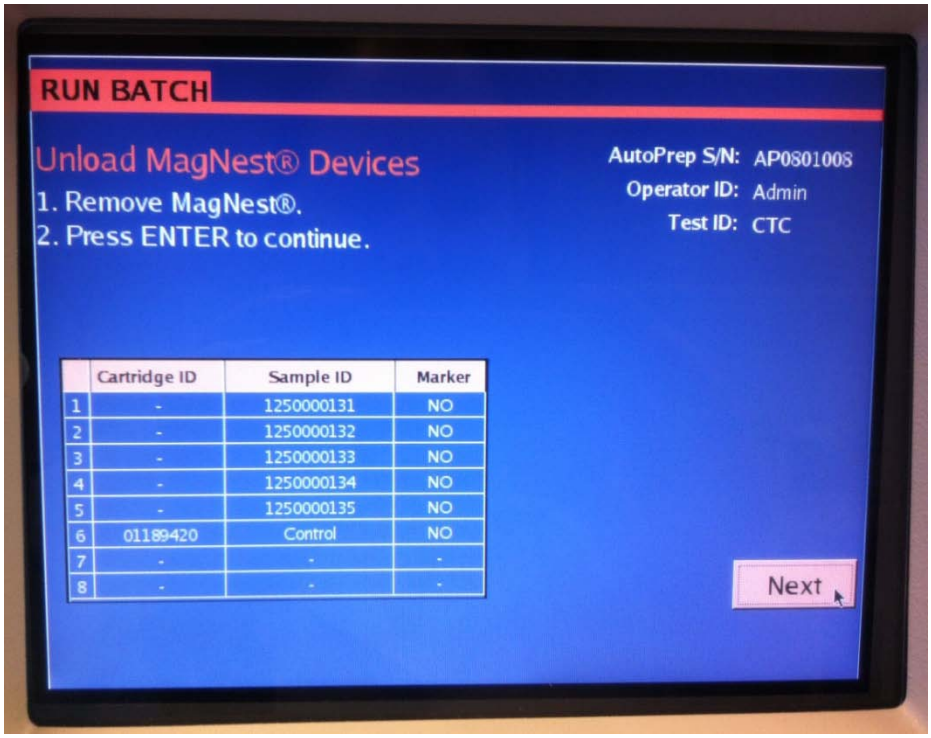
3. Sample Scanning on the Analysis Instrument

3.1 Follow the on-screen instructions on the preparation instrument to unload all samples from the system.



	Cartridge ID	Sample ID	Marker	Time Ready	Status
1	01189437	1250000131	NO	13:00	Removed
2	01189422	1250000132	NO	13:13	Removed
3	01189424	1250000133	NO	13:26	Removed
4	01189425	1250000134	NO	13:38	Removed
5	01189428	1250000135	NO	13:51	Removed
6	01189420	Control	NO	14:07	Complete
7	-	-	-	-	-
8	-	-	-	-	-

3.1.1 Upon batch completion select **Unload**.



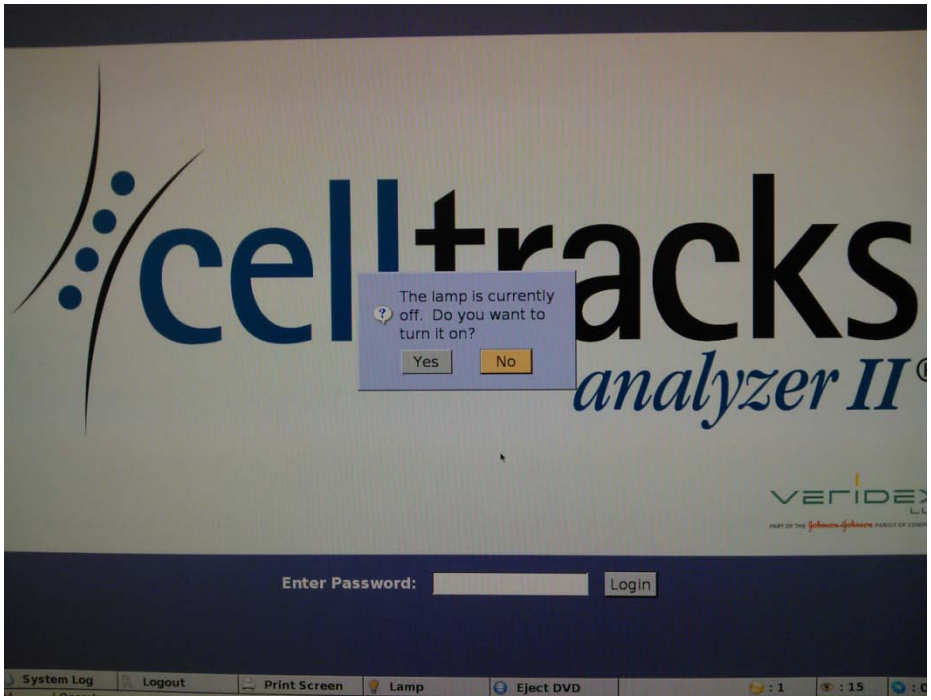
	Cartridge ID	Sample ID	Marker
1	-	1250000131	NO
2	-	1250000132	NO
3	-	1250000133	NO
4	-	1250000134	NO
5	-	1250000135	NO
6	01189420	Control	NO
7	-	-	-
8	-	-	-

3.1.2 Remove the MagNest device and click **Next**.

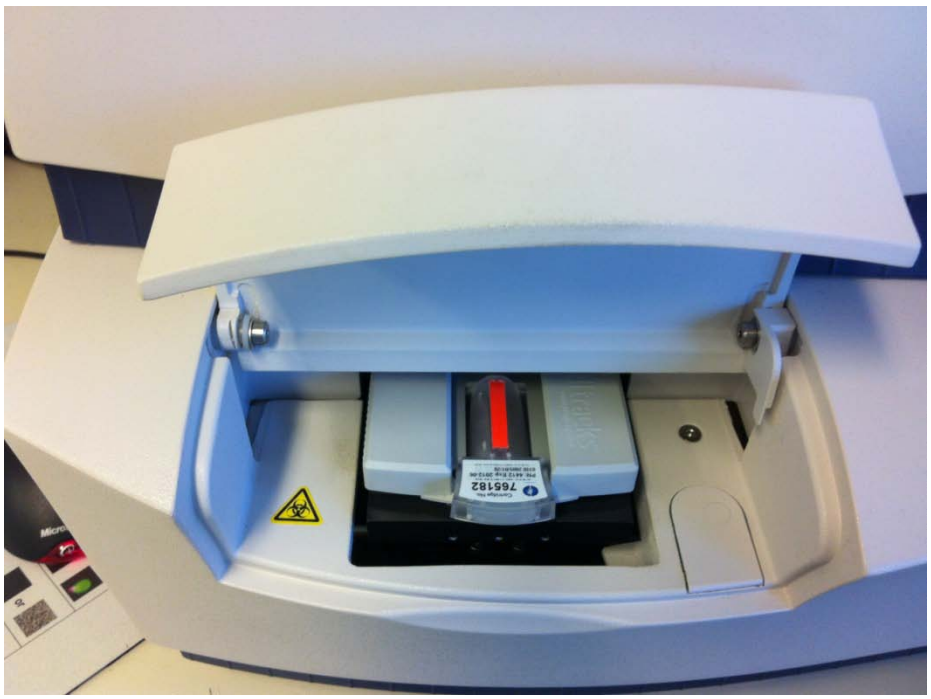
3.1.3 Remove the sample tube and click **Next**.

3.1.4 Remove the reagent kit and click **Next**.

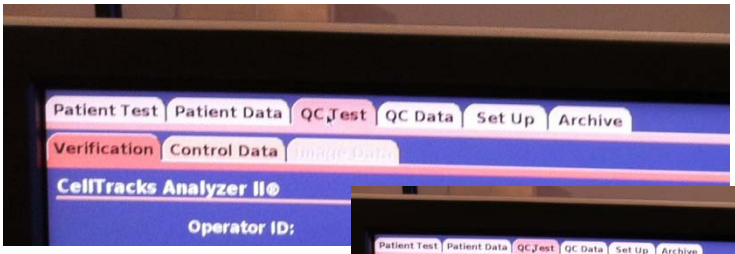
3.2 Turn on the analysis instrument and initialize the lamp. Once warmed (~ 15 minutes), load the system verification cartridge onto the analysis instrument and select the *QC Test* tab. Follow the on-screen instructions to perform the necessary quality control measures

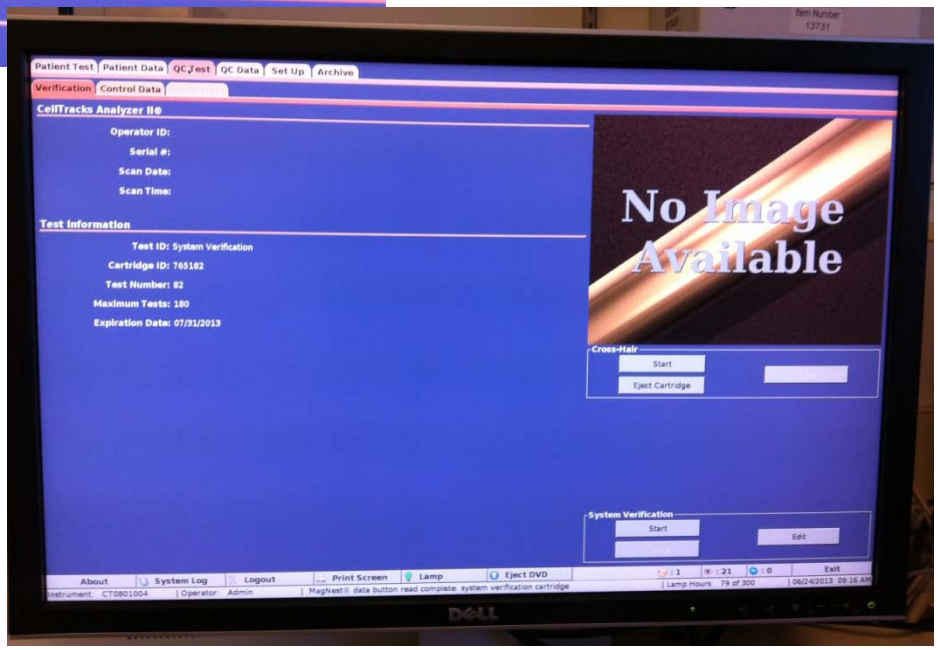


3.2.1 Select **Yes** to initialize the lamp.

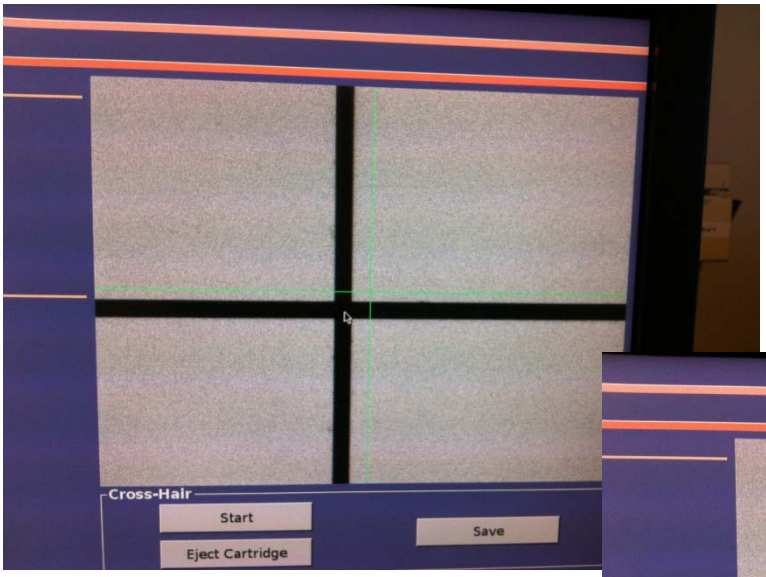


3.2.2 Load the system verification cartridge onto the analysis instrument.

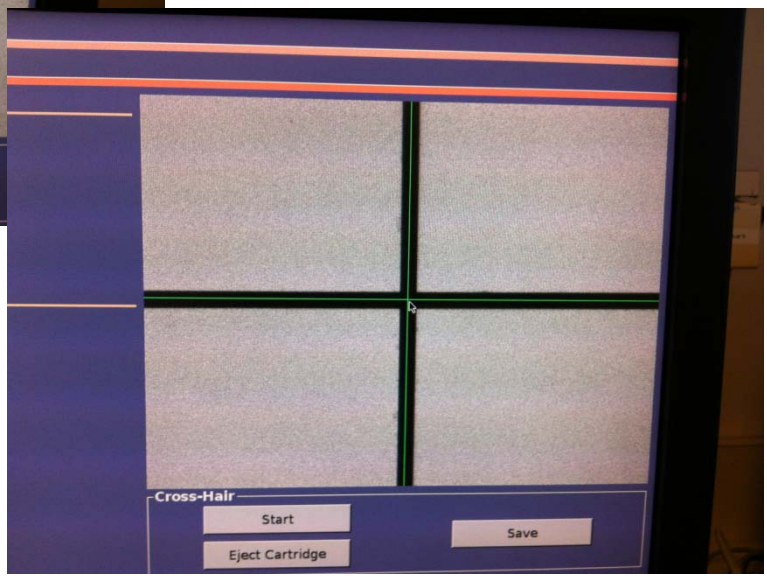


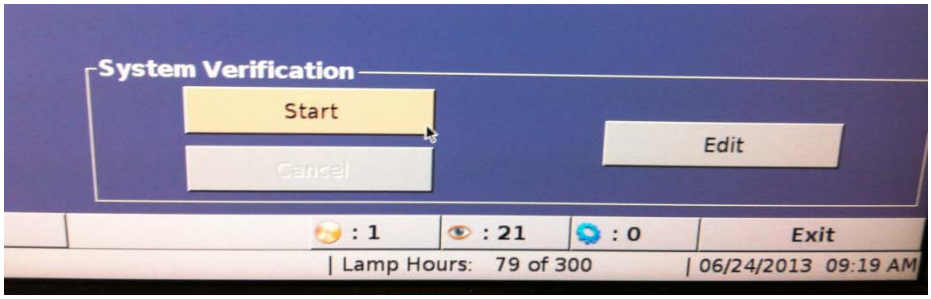


3.2.3 Select the **QC Test** Tab



3.2.4 Click **Start** under the Cross-Hair section. Adjust as necessary and click **Save**.

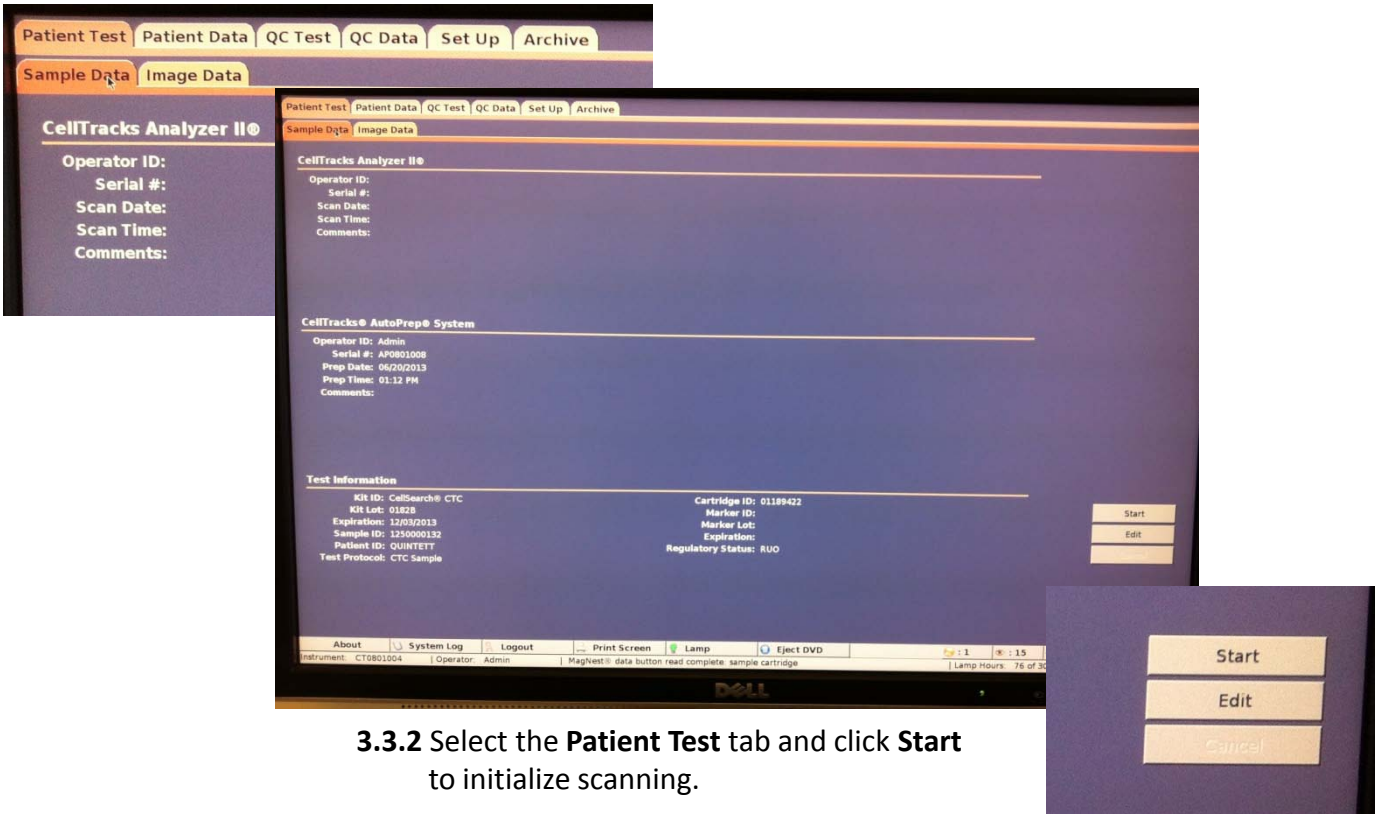




3.2.5 Click Start under the System Verification section.

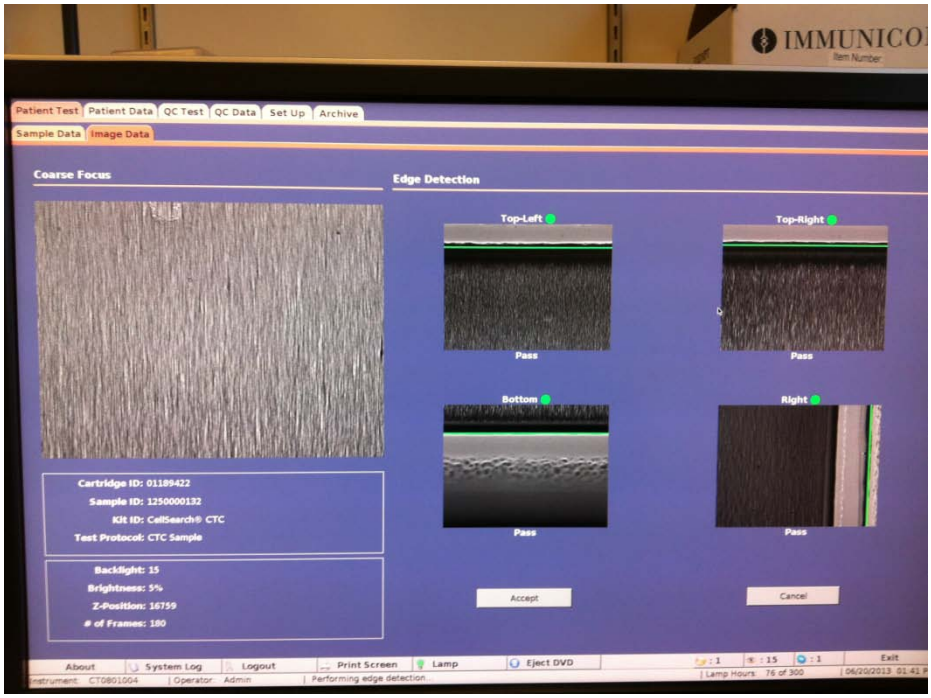
3.3 Load a sample onto the analysis instrument and select the *Patient Test* tab. All saved information from the preparation instrument will be displayed. Click *Start* to initialize sample scanning.

3.3.1 Load a sample onto the analysis instrument.

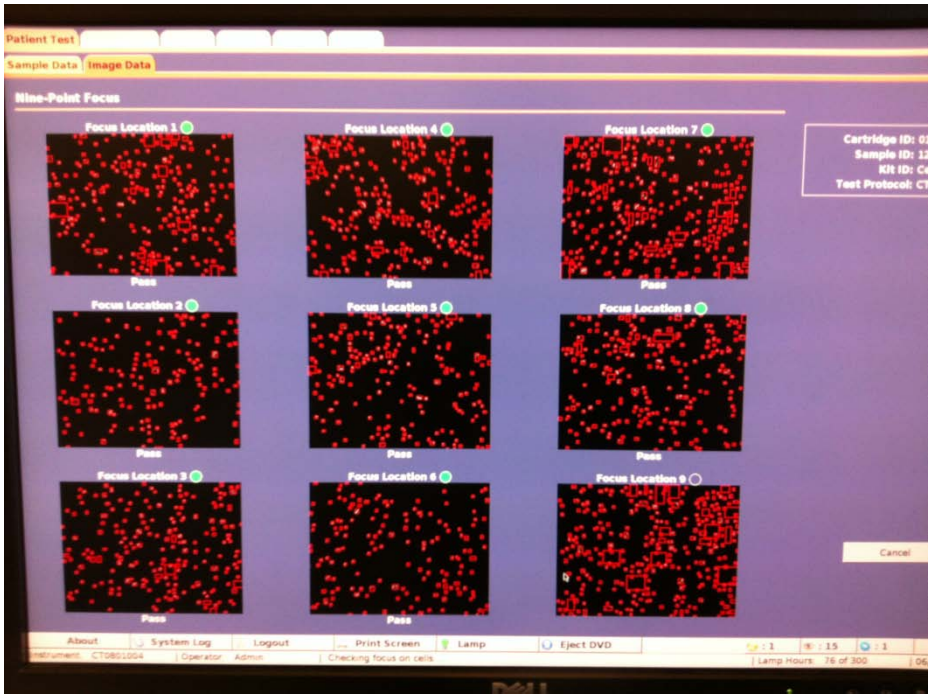


3.3.2 Select the **Patient Test** tab and click **Start** to initialize scanning.

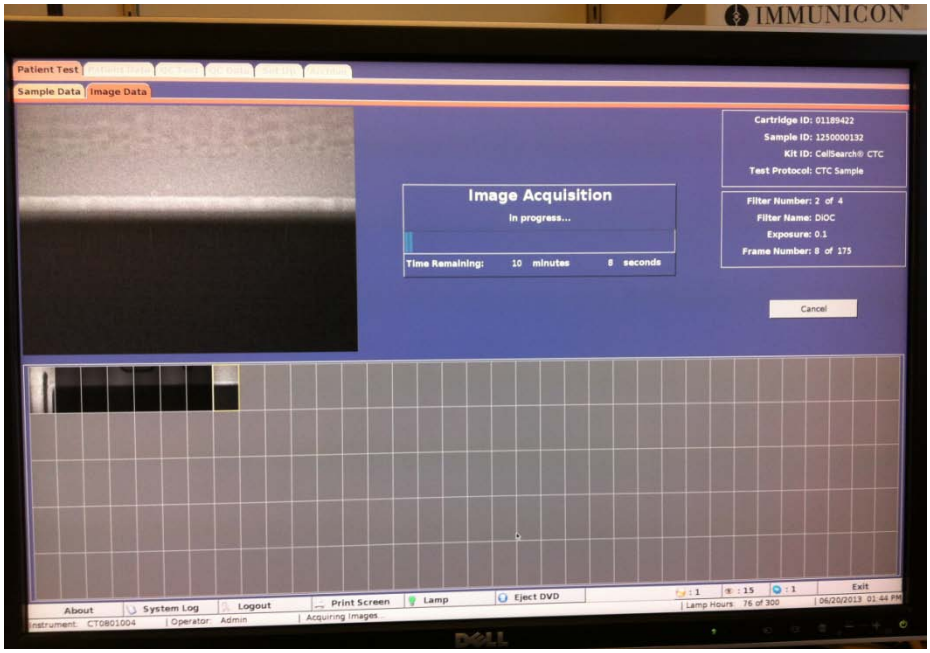
3.4 The system will perform a coarse focus and edge detection on the magnetic device cartridge. Adjust all edges as necessary using the directional keys. Select *Accept*. The system will perform a fine focus and begin sample scanning..



3.4.1 Adjust all edges as necessary using the directional keys and click **Accept**.

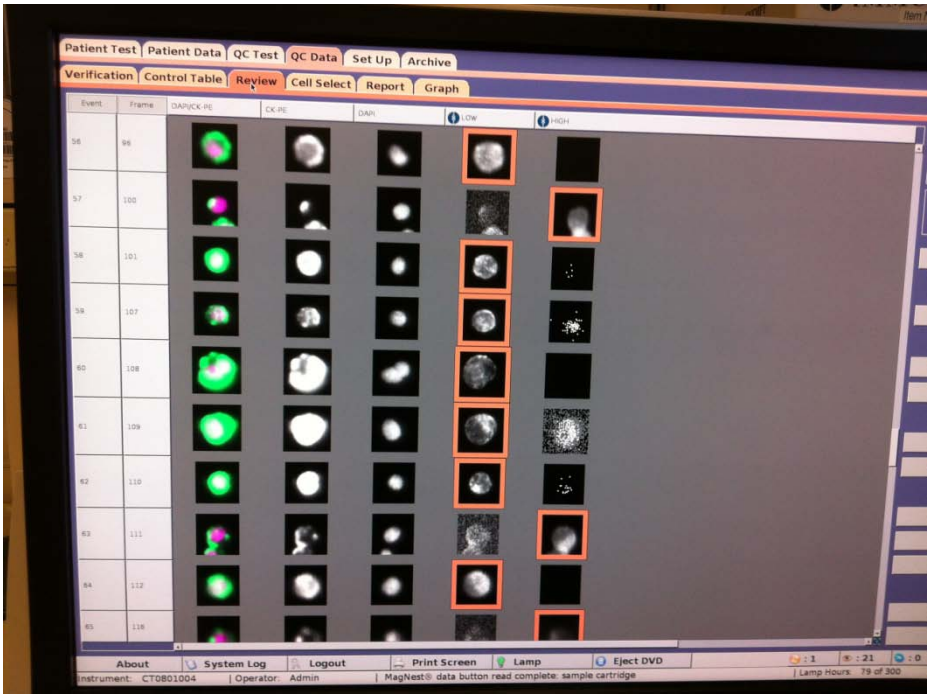


3.4.2 The system will perform a fine focus and then begin scanning.

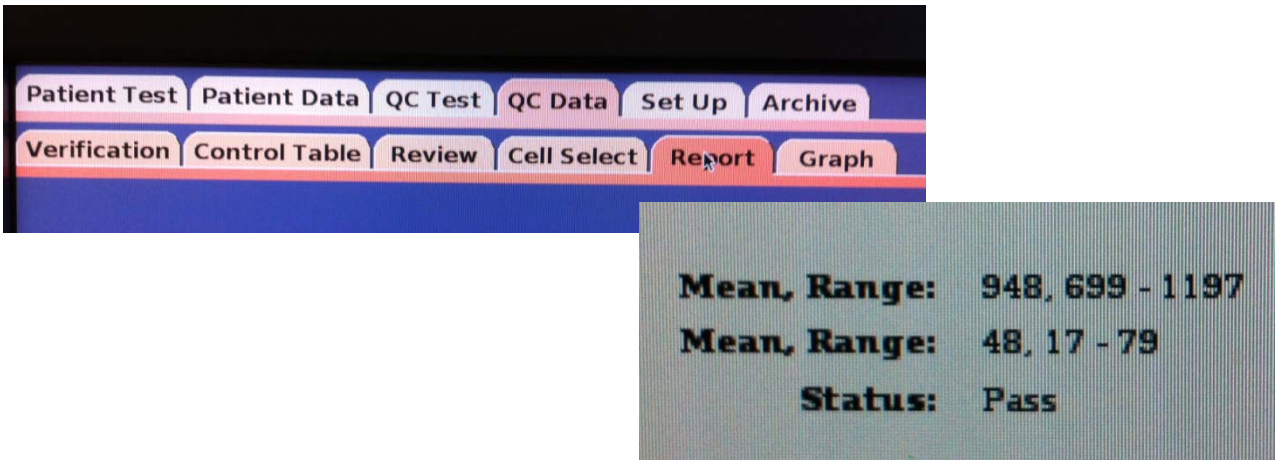


3.4.3 Automated sample scanning.

3.5 Following control scanning the results should be validated using the defined criteria for cells spiked at high ($CK^+DAPI^+CD45^-APC^+$) and low ($CK^+DAPI^+CD45^-FITC^+$) concentrations.

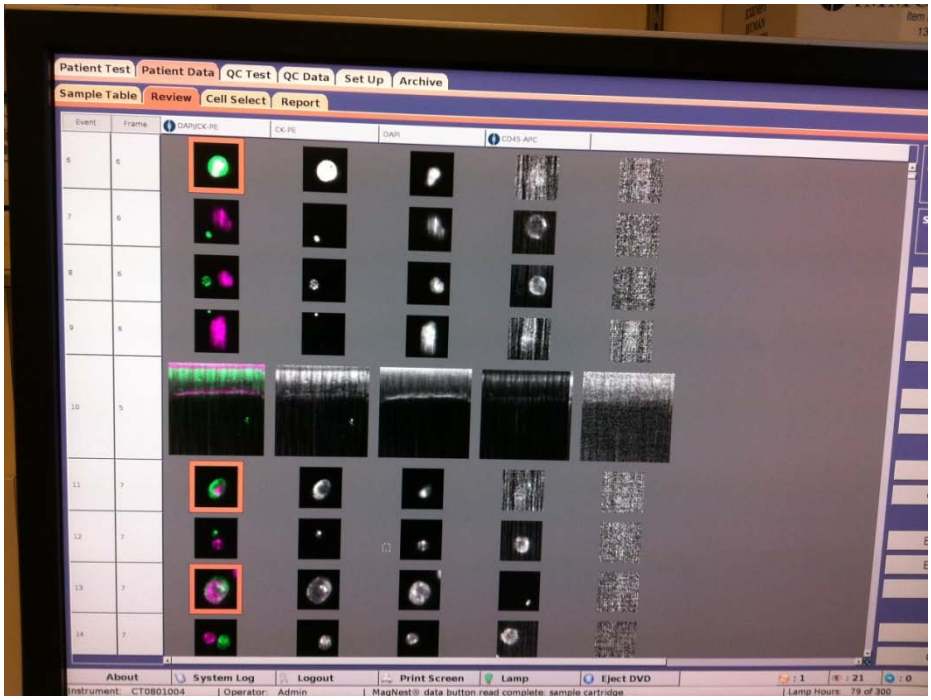


3.5.1 Selected control cells



3.5.1 Click on the **Report** tab to confirm that the control sample has passed.

3.6 Following patient sample scanning the results should be reviewed for captured CTCs using the defined CTC criteria ($CK^{+}DAPI^{+}CD45^{-}$)

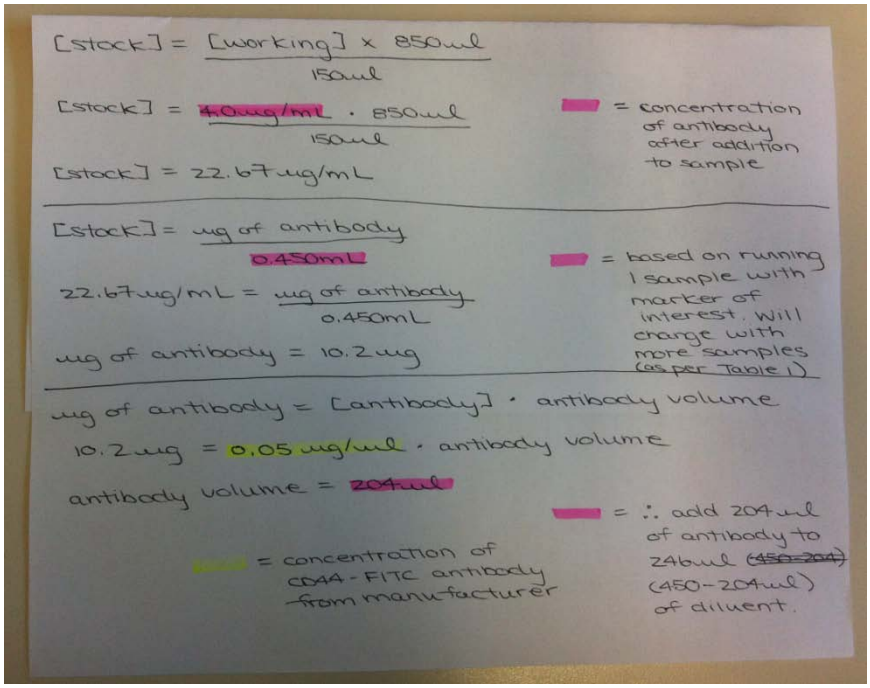


3.6.1 Selected CTCs.

CTC Characterization for User-Defined Markers using the CSS

1. Preparation of User-Defined Markers and Instrument Initialization

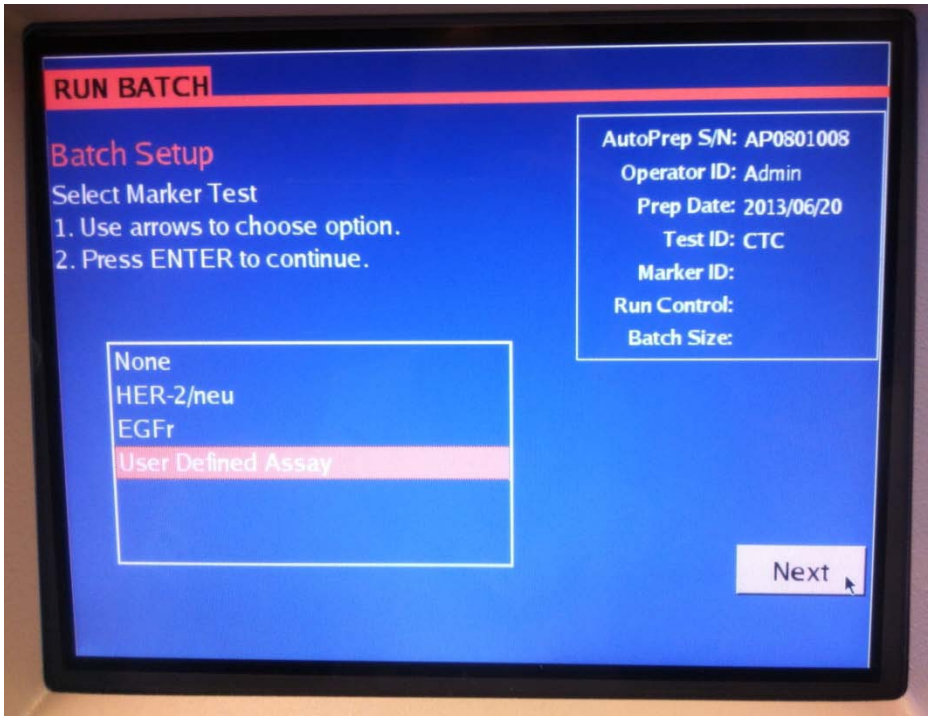
1.1 Dilute the antibody of interest using Bond Primary Antibody Diluent to the desired concentration in a marker reagent cup. Place the marker reagent cup into position 1 in the reagent cartridge and load the cartridge onto CSS.



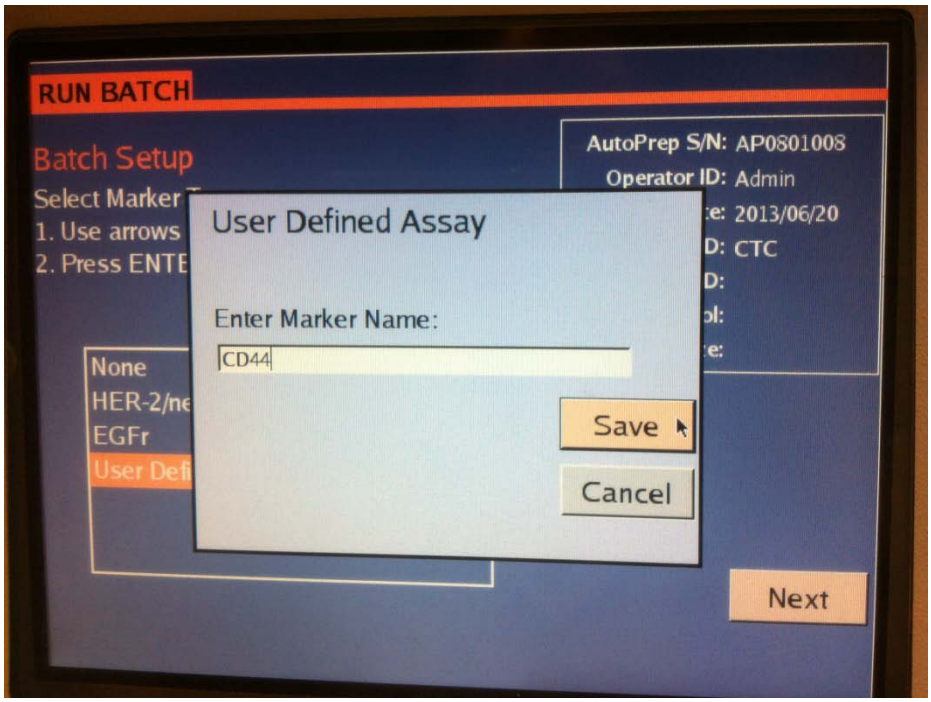
1.1.1 Antibody dilution calculations for a 4.0µg/ml working concentration for 1 sample using CD44-FITC (stock concentration = 0.05µg/µl) as an example.

1.1.2 Place the marker reagent cup into position 1 in the reagent cartridge.

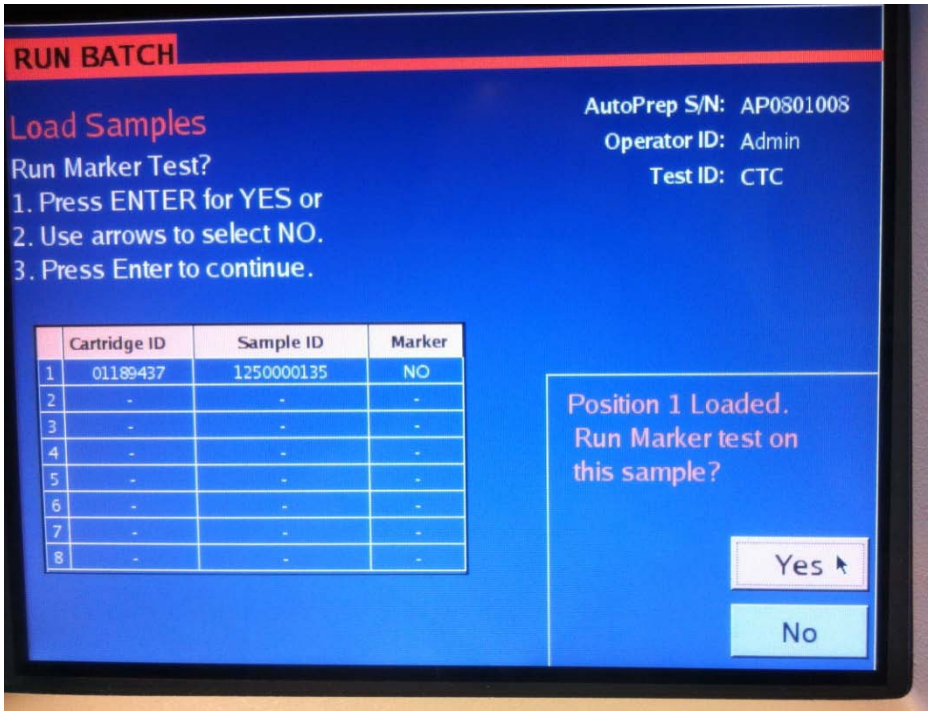
1.2 Collect blood, prepare samples, and load the preparation instrument as described in the above ***Standard CTC Enumeration from Patient Blood Samples using the CSS*** protocol. To enable custom marker addition, select *User Defined Assay* when prompted by the preparation instrument. Input the marker name and select *Save*. As samples are loaded onto the, you will prompted to indicate which should receive custom marker by selecting *Yes* or *No* as necessary.



1.2.1 Select **User Defined Assay** and click **Next**.



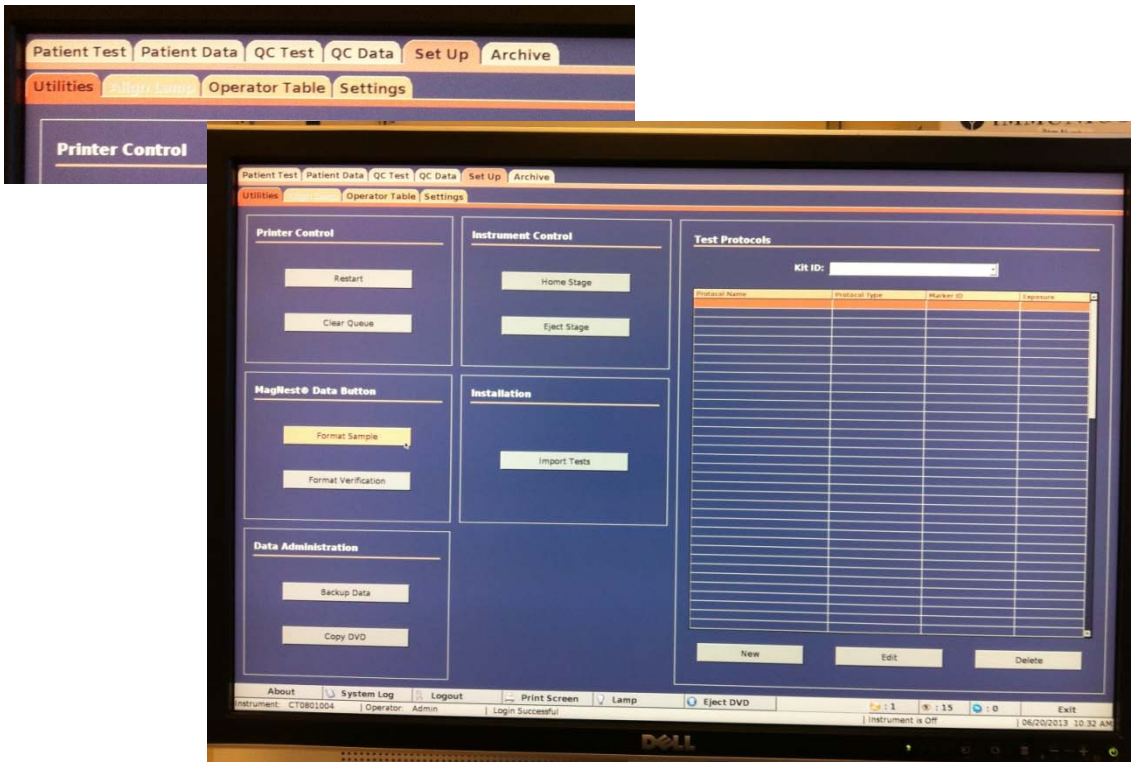
1.2.2 Enter the marker name, select **Save** and click **Next**.



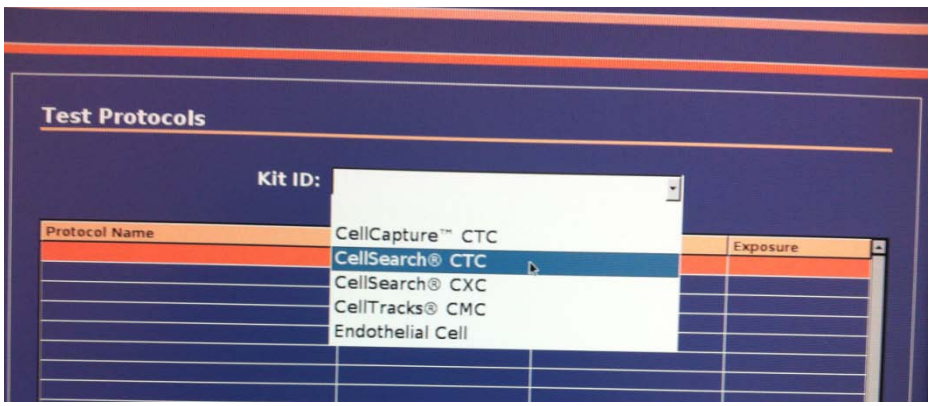
1.2.3 When prompted indicate which samples should receive custom marker by selecting **Yes** or **No** as necessary.

2. Sample Scanning of User-Defined Markers on the Analysis Instrument

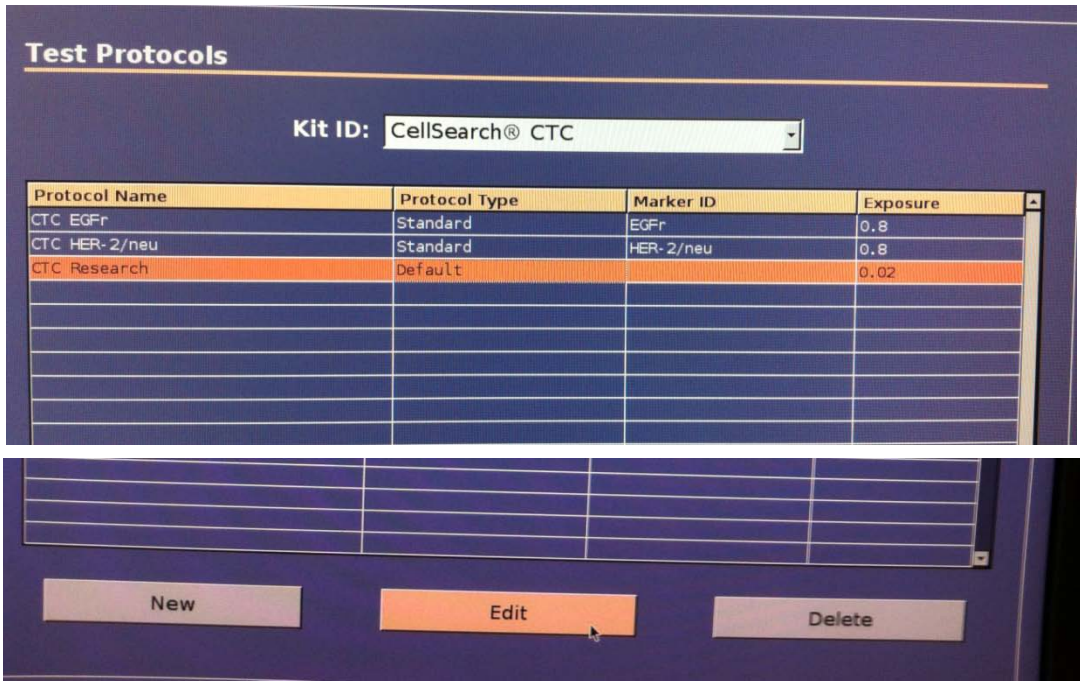
2.2 Load a sample onto the analysis instrument and select the *Setup* tab. To initialize the FITC channel, select *CellSearch CTC* as the *Kit ID* under the *Test Protocols* section. From this menu, select *CTC Research*, click the *Edit* button and set the exposure time as desired. It is recommended that an exposure time of 1.0sec not be exceeded when using the CSS CTC kit as this can increase bleed-through into other fluorescent channels utilized for CTC identification.



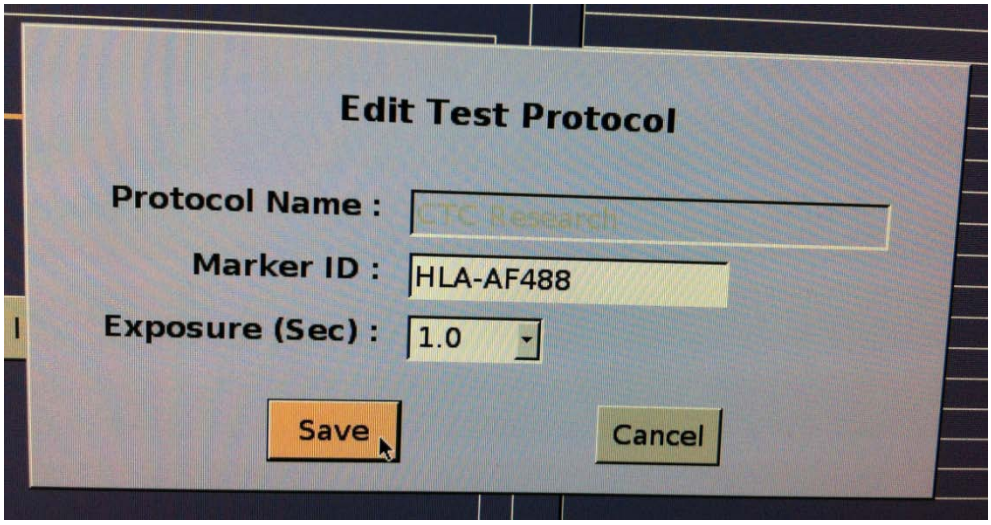
2.2.1 Select the **Setup** tab.



2.2.2 Select **CellSearch CTC**.



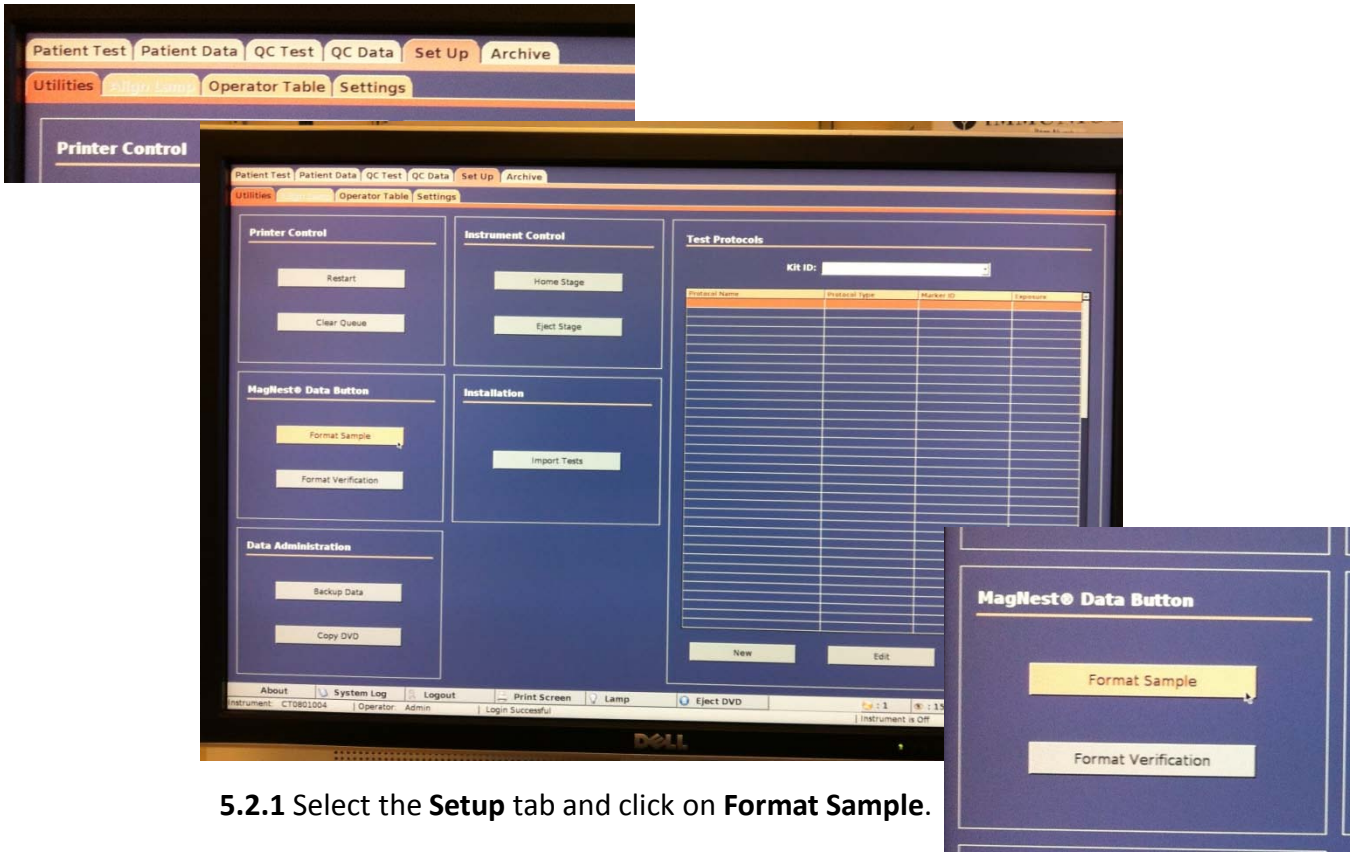
2.2.3 Select **CTC Research** and click **Edit**.



2.2.4 Select exposure time and click **Save**.

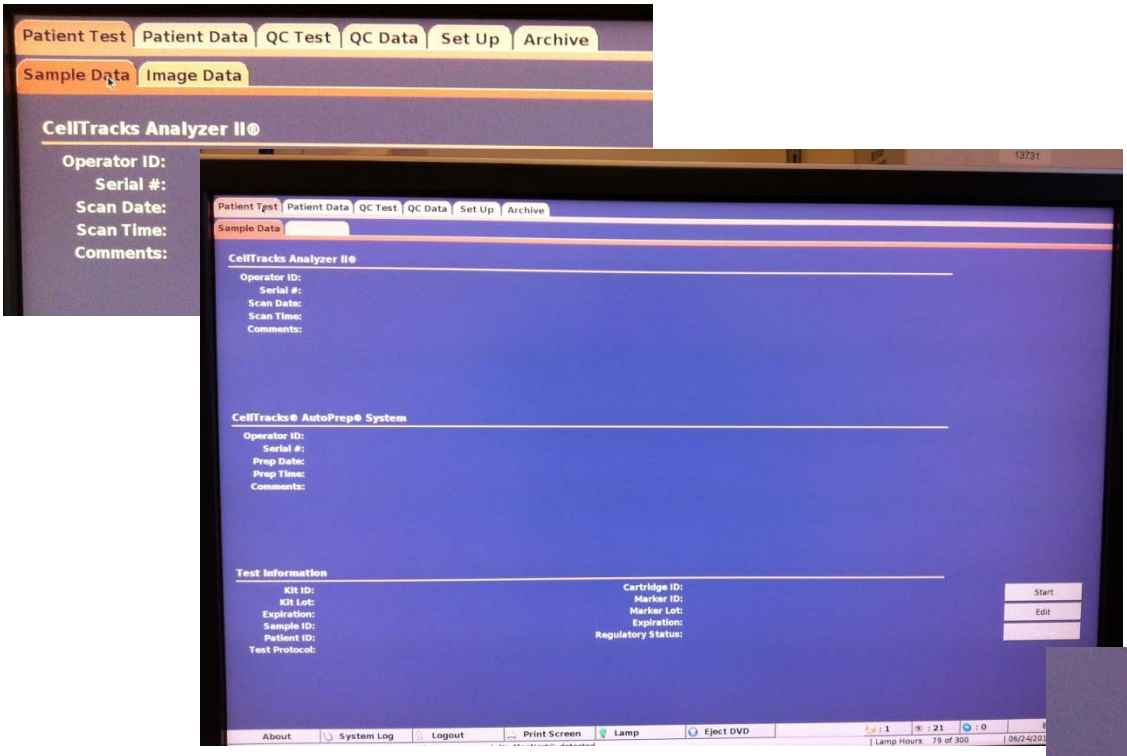
5. Scanning of Manually Separated Samples on the Analysis Instrument

5.2 Load the sample onto the analysis instrument and select the *Setup* tab. Clear any existing data on the magnetic device data button by clicking the *Format Sample* button. Enable the FITC channel and set the exposure time to 1.0 sec as described in section 2.2 of the ***CTC Characterization for User-Defined Markers using the CSS***.

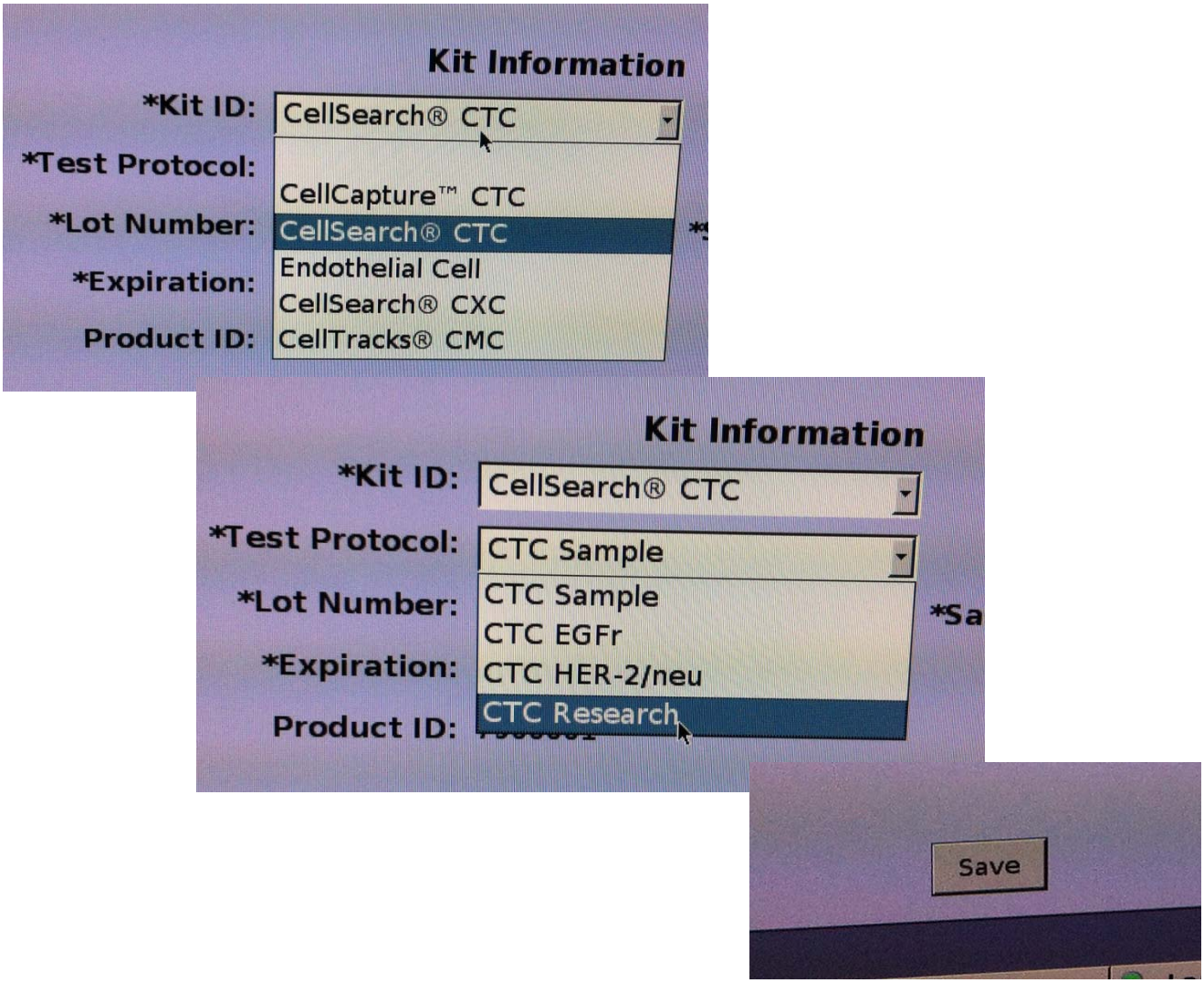


5.2.1 Select the **Setup** tab and click on **Format Sample**.

5.3 Click on the *Patient Test* tab and select *Edit* to input the sample information. Select *CellSearch CTC* as the *Kit ID* and *CTC Research* as the *Test Protocol*. Input the remaining necessary information as indicated as the asterisk. *Save* the sample information and click *Start*.



5.3.1 Select the **Patient Test** tab and select **Edit**.



5.3.2 Select **CellSearch CTC** as the Kit ID and **CTC Research** as the Test Protocol. Click **Save**.