

DCTD Standard Operating Procedure (SOP)

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Doc. #:	LHTP003.8.1	Revision:	E	Effective Date:	5/6/2013

Pharmacodynamic Assay Development and Implementation Section (PADIS)

Applied Developmental Directorate

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Change History

Revision	Approval Date	Description	Originator	Approval
--	9/28/2009	New Document	LW, TP	RJK
A	7/07/2010	Format SOP, add Batch Record, and Appendices	YAE	RJK
B	12/23/2010	Updates based on first training course	LW, TP	RJK
C	9/13/2011	Removed shipping instructions for off-site blood processing and referenced SOP LHTP003.08.03 for blood collection and shipping. Section added to Batch Record to record MagNest information.	LW	RJK
D	3/26/2012	Removed specific references to use of the γ H2AX-AF488 antibody. Generalized SOP for use with any qualified primary antibody conjugated to a FITC (or similar) reporter.	YAE	RJK
E	5/6/2013	Batch Record and SOP updated to remove redundant data elements from the Batch Record that can be input into the CellSearch system and are printed as part of the CellTracks Research Report. An example Research Report added as an Appendix.	YAE, LW	RJK

Please check for revision status of the SOP at

<http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>

and be sure to use the current version.



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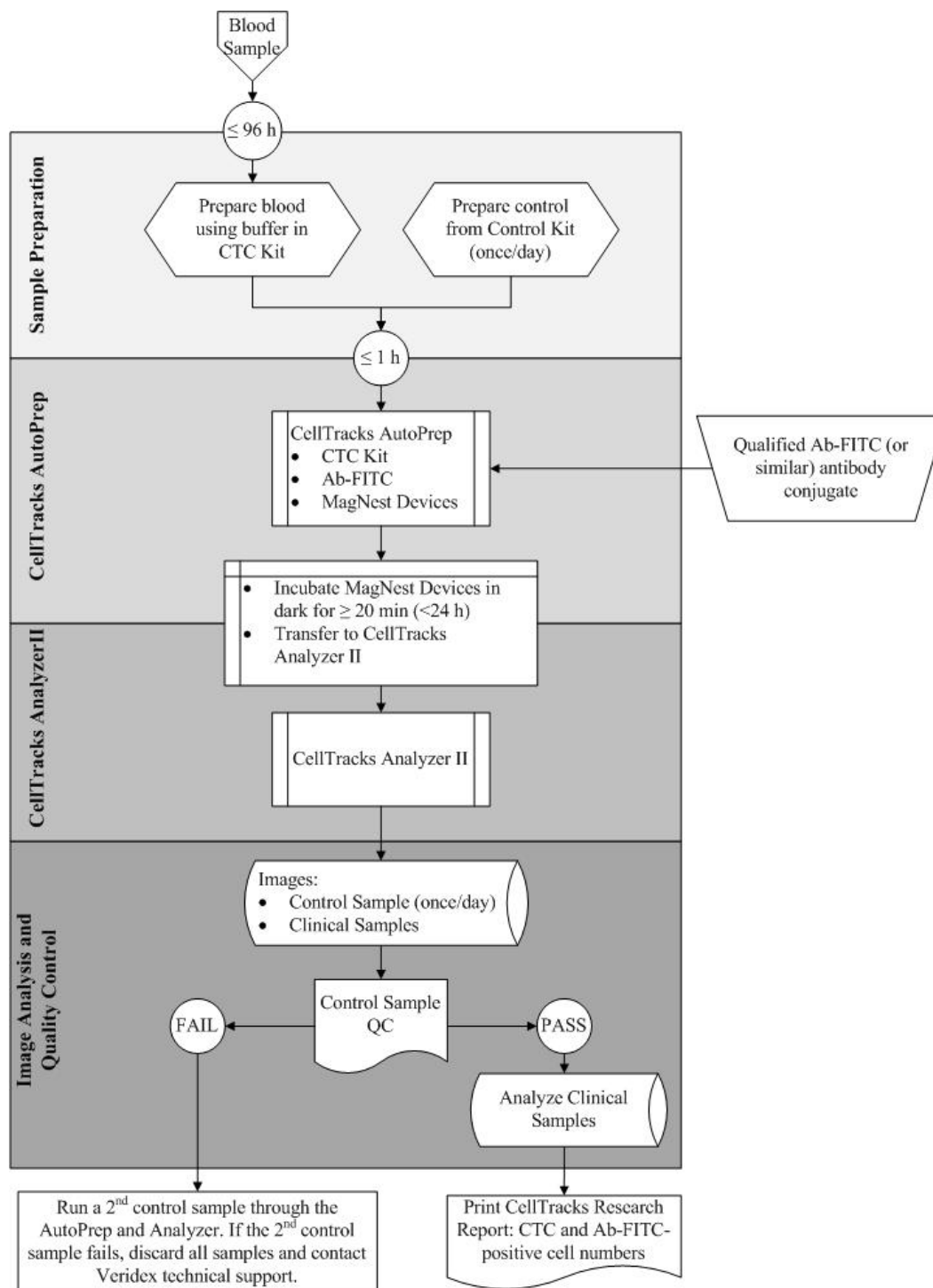
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OVERVIEW OF ASSAY SAMPLE PROCESSING



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1.0 PURPOSE

To standardize the method for immunomagnetic selection of circulating tumor cells (CTCs) and immunofluorescent identification of CTCs positive for pharmacodynamic (PD) biomarkers, nuclear or cytoplasmic, as measures of chemotherapeutic agents.

2.0 SCOPE

This procedure applies to all personnel responsible for the collection and processing of clinical samples for biomarker analysis of CTCs using a qualified primary antibody conjugated to FITC (or similar; Ab-FITC), and those responsible for performing the Immunofluorescence assay (IFA) for CTCs on these samples using the CellSearch[®] System CellTracks[®] AutoPrep[®] and Analyzer II. The goal of the SOP and associated training is to ensure consistency of biomarker measurement for CTCs between clinical sites.

3.0 ABBREVIATIONS

Ab	=	Antibody
Ab-FITC	=	Primary Antibody Conjugated to FITC (or similar) Reporter
CTC	=	Circulating Tumor Cell
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
DI	=	Deionized
FITC	=	Fluorescein Isothiocyanate or Similar Reporter (e.g., AF488)
IFA	=	Immunofluorescence Assay
LHTP	=	Laboratory of Toxicology and Pharmacology
PADIS	=	Pharmacodynamic Assay Development and Implementation Section
PD	=	Pharmacodynamic
PHL	=	Pathology/Histotechnology Laboratory
QC	=	Quality Control
RT	=	Room Temperature
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The IFA for CTCs is an immunohistochemistry-based staining assay developed to quantify CTCs positive for a biomarker of interest using a qualified antibody conjugate. The assay uses a qualified primary antibody conjugated to FITC (or similar) reporter for immunofluorescent identification of cells. CTCs are enriched from whole blood and isolated using the CellSearch System, an automated system for the purification and enumeration of CTCs with the capability of detecting a single CTC in 7.5 mL of whole blood.

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5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor The Laboratory Director/Supervisor, directs laboratory operations, supervises technical personnel, reporting of findings, and is responsible for the proper performance of all laboratory procedures. The Laboratory Director/Supervisor oversees the personnel running SOPs within the laboratory and is responsible for ensuring this person(s) is certified and has sufficient experience to handle clinical samples.

Laboratory Scientist A Laboratory Scientist should have at least a PhD and 3 years or an MS and 5 years of experience in assay validation and have worked in a clinical testing laboratory setting. Laboratory Scientists supervise Laboratory Technicians, review SOP results, perform assay steps requiring specialized scientific skills, and, in the absence of the Laboratory Supervisor, is responsible for the proper performance of all laboratory procedures. The Laboratory Scientist is also responsible for monitoring Batch Record preparation and following-up on any issues that arise during SOP performance that qualify as a deviation.

Certified Assay Operator A Certified Assay Operator **must be** a Laboratory Scientist who has been certified through DCTD training on this SOP. The Certified Assay Operator works under the guidance of the Laboratory Director/Supervisor. This person, in accordance with the current SOP(s), performs laboratory procedures and examinations and any other procedures conducted by a laboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

- 5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all Certified Assay Operator meet the criteria for the role defined as Laboratory Scientist.
- 5.2 It is the responsibility of the Laboratory Director/Supervisor to ensure that the Certified Assay Operator has received training by Veridex, LLC in the use, operation, and maintenance of the CellSearch System, including the CellTracks AutoPrep System and the CellTracks Analyzer II. Veridex certification is **required** for performance of this SOP. In addition the Certified Assay Operator must have documented DCTD training and certification on this SOP prior to the actual handling and processing of specimens from clinical trial patients.
- 5.3 It is the responsibility of the Laboratory Director/Supervisor to ensure that the Certified Assay Operator qualifies all new batches of Ab-FITC conjugate and the laboratory completes laboratory proficiency testing during initial laboratory set up, any time a new certified assay operator is added to the laboratory, and each time a new batch of Ab-FITC conjugate is qualified. As an example, SOP LHTP003.8.1.1 for qualification of γ H2AX-AF488 is available on the [DCTD Biomarkers Web site](#) as a requirements document for community-provided antibody qualification methods.
- 5.4 It is the responsibility of the Laboratory Director/Supervisor of the specimen collection laboratory to confirm scheduled specimen collection time points, print all labels in advance, check documentation for accuracy, and verify that the required collection tubes, supplies, and equipment are available for successful isolation and preparation of CTCs.

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- 5.5 Certified Assay Operators following this SOP are required to be certified in working safely with bloodborne pathogens in research laboratories in accordance with OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030).
- 5.6 It is the responsibility of the Certified Assay Operator to ensure timely transport and processing of the samples, enter and review all of the required collection and processing data, and archive all data sheets in the appropriate files.
- 5.7 Digital versions of the Batch Record (Appendix 1, Sections 1 and 2) can be created for logging information as long as all column and row information exactly matches that in the Batch Record. If any portion of the Batch Record is recorded in a digital copy, cross through that section, state "See attached sheet," and initial. A copy of the completed, digital table must be printed and attached to the Batch Record in order to maintain a complete audit trail.
- 5.8 The Certified Assay Operator responsible for carrying out the assay is to follow this SOP and complete the required tasks and associated documentation. The Batch Record (Appendix 1) must be completed in **real-time** for each experimental run, with each page ***dated and initialed***, and placed with the clinical sample information.
- 5.9 The Certified Assay Operator is to check the DCTD Biomarkers Web site (<http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>) to verify that the latest SOP version is being followed.

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6.0 MATERIALS AND EQUIPMENT REQUIRED

- 6.1 Pipettors (100-1000 µL, 50-200 µL) and tips
- 6.2 Electronic pipette
- 6.3 1-, 5-, 10-, and 25-mL pipettes, sterile, individually wrapped (Fisher Scientific, Cat#: 13-675-15C, 13-675-22, 13-675-20, and 13-668-2)
- 6.4 1.5-mL Sarstedt o-ring screw cap tubes (Fisher Scientific, Cat#: 72.692.005)
- 6.5 50-mL polypropylene tubes (Becton Dickinson, Cat#: 352098)
- 6.6 Lens paper (e.g., Fisher Scientific, Cat#: 11-996)
- 6.7 DVD+R, Veridex recommends Memorex 120 min, 4.7 GB, 16x
- 6.8 Clorox Bleach ultra (6.15% hypochlorite) or regular (5.25%); alternative bleach products must be filtered according to Veridex, LLC specifications. Dilute 85 mL ultra or 99 mL regular bleach to a final volume of 2 L in distilled water to prepare a 0.26% solution for use in the assay.
- 6.9 Deionized (DI) water, sterile
- 6.10 Qualified primary antibody conjugated to FITC (or similar; Ab-FITC). Examples of FITC-similar dyes include Alexa Fluor 488 and DyLight 488.
- 6.11 Bond Primary Antibody Diluent (Leica Microsystems, Cat#: AR9352)
- 6.12 CellTracks AutoPrep System (Veridex, Cat#: 9541); system includes 8 MagNest® devices and reagent carousel
- 6.13 CellTracks Analyzer II (Veridex, Cat#: 9555)
- 6.14 MagNest Device, blue cover, 1-pack (Veridex, Cat#: CT4247)
- 6.15 CellSave Preservative Tubes, 100-pack (Veridex, Cat#: 7900005)
- 6.16 CellSearch Printer Paper (Veridex, Cat#: CT1828)
- 6.17 CellSearch Circulating Tumor Cell Kit (Veridex, Cat#: 7900001)
- 6.18 CellSearch Circulating Tumor Cell Control Kit (Veridex, Cat#: 7900003)
- 6.19 CellTracks System Buffer, 20 L cubitainer (Veridex, Cat.# 7901003)
- 6.20 CellTracks System Verification Cartridge, 2-pack (Veridex, Cat#: CT9566)
- 6.21 Sorvall Legend RT centrifuge (Fisher Scientific, Cat#: 75-006-434) with a swing bucket rotor
- 6.22 Vortex-Genie (Scientific Industries, Inc.)
- 6.23 Monthly maintenance kit (Veridex, Cat#: 9554)
- 6.24 Class II Type A2 biosafety cabinet/tissue culture hood
- 6.25 4°C refrigerator
- 6.26 Optional: SOP LHTP003.08.03: CellSave Tube: Human Blood Collection and Specimen Submission. Provided during Certified Assay Operator training and available on the [DCTD Biomarkers Web site](#)

* If instruments and/or reagents differ from those specified above, the Certified Assay Laboratory performing the assay must prove their comparability or equivalence to those recommended using the manufacturer's specifications and experimental validation data.

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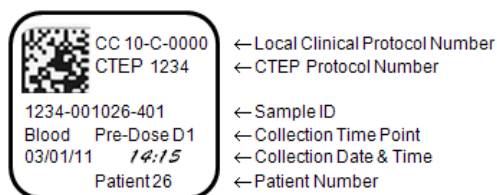
7.0 OPERATING PROCEDURES

Note: The general blood collection and processing procedures follow the CellSearch CTC Kit–recommended protocol.

- 7.1** All new batches of Ab-FITC conjugate need to be qualified for use with the Cell Search System before using this SOP. As an example, SOP LHTP003.8.1.1 for qualification of γ H2AX-AF488 is available on the [DCTD Biomarkers Web site](#) as is a requirements document for community-provided antibody qualification methods.
- 7.1.1** In addition, all new Certified Assay Operators in a laboratory are required to complete proficiency testing associated with the antibody qualification SOP prior to running clinical samples in the laboratory.
- 7.1.2** Because a single lot of antibody may last a laboratory several years, it is recommended to re-validate a lot of antibody every 6 months to verify that the antibody still meets assay performance.
- 7.2** Record the name, and DCTD certification number of the Certified Assay Operator in the Batch Record (Appendix 1). Be sure to review all actions required for successful assay setup.
- 7.3** Two identical, pre-printed specimen labels should be prepared for each blood collection time point. Labels should include Sample ID, clinical trial number, collection time point, collection date, and space to write the actual collection time.

The Sample ID should be a unique alpha-numeric identifier; it is recommended that the ID include the CTEP protocol number followed by the patient identifier and a unique specimen ID.

NCI collections for CTC PD sampling include a unique specimen ID using series 400 with consecutive numbers identifying the collection time points as defined in the Clinical Protocol. Non-NCI locations may use unique specimen IDs such as the defined collection time points (e.g., C1D1-pre, C1D8-12h). Example pre-printed label:



7.4 Blood Collection

- 7.4.1** Blood sample collection laboratory personnel should ensure that the phlebotomist is using the recommended CellSave Preservative Tube to draw the blood samples. If necessary, supply the phlebotomist with the correct tubes. A minimum of 7.5 mL of blood should be collected into the tubes.
- 7.4.2** The research nurse is to notify the laboratory of scheduled PD specimen collections, preferably giving at least 24-h notice. A laboratory technician is to arrive at the blood collection site at least 5 min ahead of the scheduled time point(s) to ensure rapid transport to the laboratory after collection.
- 7.4.3** Place one of the pre-printed labels onto the freshly collected CellSave Preservative Tube and give the second label to the research nurse to place in the patient record sheet.
- 7.4.4** Samples should be mixed immediately by gently inverting the tube 8 times to prevent clotting. Write the actual time of blood sample collection on the specimen label.

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7.4.5 Samples should be kept at RT (15°C to 25°C); do not place on ice or refrigerate. Patient blood samples are stable for 96 h in CellSave Preservative Tubes at RT.

7.4.6 If shipping blood to PADIS for processing, follow SOP LHTP003.08.03: CellSave Tube: Human Blood Collection and Specimen Submission provided during Certified Assay Operator training and available on the [DCTD Biomarkers Web site](#); otherwise, store blood samples at RT for no longer than 96 h prior to sample preparation following SOP Step 7.5.

7.4.6.1 If shipping blood samples to a secondary site other than PADIS for PD biomarker analysis, follow the same general guidelines outlined in SOP LHTP003.08.03.

7.5 Sample Preparation

7.5.1 All reagents and kits should be labeled with date of receipt and date opened. Reagents should be stored under the specified conditions for no longer than the recommended duration.

7.5.2 Upon receipt of blood samples from the clinic, verify that each patient sample is labeled with a unique Sample ID, clinical trial number, collection time point, collection date, and collection time. If any of this information is missing, notify the Laboratory Supervisor, and they will contact the clinical monitor to obtain the missing information.

Important: Patient blood samples are stable for up to 96 h after collection in CellSave Preservative Tubes at RT (15°C to 25°C). **Do not** place on ice or refrigerate.

7.5.3 Remove the CellSearch CTC Kit and CTC Control Kit (if needed) from the refrigerator 30 min prior to use and allow it to warm to RT.

7.5.3.1 One control sample from the Control Kit needs to be run and pass QC on **each day** of clinical sample testing and each time a new lot of the CTC Kit is used.

7.5.3.2 For one experimental run, a maximum of 8 samples (including control) can be processed.

7.5.4 The first sample run each day should be a control sample

7.5.4.1 If a control sample has already passed QC for the day, indicate “Yes” in the Batch Record (Appendix 1, Section 1) and skip to SOP Step 7.5.6.

7.5.4.2 If a control sample has not yet passed QC for the day, indicate “No” in the Batch Record (Appendix 1, Section 1) and proceed to SOP Step 7.5.5. Once a control sample passes QC during this assay run, indicate this by placing your initials at the top of the Sample Information Table as indicated (Appendix 1, Section 1).

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7.5.5 Control Sample – Control Kit (once/day)

- 7.5.5.1 If a control sample has not yet been run for the day, run a control sample as the last tube in the first run and record the information for the tube in the Sample Information Table of the Batch Record (Appendix 1, Section 1).
- 7.5.5.2 From the Control Kit, affix a supplied orange barcode label vertically to one of the provided 15-mL AutoPrep tubes and place in a tube rack. **Note:** The barcodes are lot-specific and should not be used with other Control Kits.
- 7.5.5.3 Vortex one of the supplied 3-mL control bottles gently for 5 sec and then mix by inverting 5 times.
- 7.5.5.4 Pour the contents of the control bottle into the AutoPrep tube. Use a pipettor to ensure any residual liquid is transferred to the AutoPrep tube.

7.5.6 Clinical Sample(s) – CTC Kit

Clinical sample preparation must begin within 96 h of blood collection.

- 7.5.6.1 Record the Patient/Sample ID in the Sample Information Table of the Batch Record (Appendix 1, Section 1). The order the tubes are recorded in the table should be the order the tubes are processed in the AutoPrep.

For EACH sample, the following information will be entered into the **CellTracks AutoPrep** (or Analyzer) in SOP Step 7.6.5.7:

- Patient/Sample ID (AutoPrep only)
 - Blood draw date and time (AutoPrep only)
 - CTEP# and/or Clinical Protocol number
 - Collection location (e.g., NCI CC)
 - Deviations and notes on samples.
- 7.5.6.2 For each patient sample, label one of the provided 15-mL AutoPrep tubes with the Sample ID. Place the AutoPrep tubes in a tube rack, in order, according to the Sample Information Table.
 - 7.5.6.3 Using a 10-mL pipette, transfer 7.5 mL of blood from a CellSave Preservative Tube into the corresponding labeled 15-mL AutoPrep tube, and then add 6.5 mL Dilution Buffer (15°C to 25°C, included with the CTC Kit); be sure buffer has reached RT before addition.

Discard any remaining blood and the CellSave Preservative Tubes in the appropriate biohazardous waste containers.
 - If a sample is processed and it has been > 96 h since collection or if the blood sample contains < 7.5 mL, note the deviation in the Comments field of the CellTracks Analyzer or the Batch Record (Appendix 1, Section 3).
 - 7.5.6.4 Cap the AutoPrep tubes, mix by inversion 5 times, and then centrifuge the samples in a swing bucket rotor at 800 x g for 10 min at 15°C to 18°C, without the brake.
 - 7.5.6.5 Samples must be loaded into the CellTracks AutoPrep System **within 1 h** of sample preparation. **Do not** place on ice or refrigerate.

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7.6 CellTracks AutoPrep System

7.6.1 If the AutoPrep is off, turn it on and let it warm up for 30 min prior to use.

7.6.2 Be sure the following solution bottles are filled and attached:

7.6.2.1 Waste bottle – empty and then fill with 400 mL DI water

7.6.2.2 Cleaning solution bottle – fill with 0.26% bleach

7.6.2.3 Instrument buffer bottle – System Buffer, refill using 20-L cubitainer

7.6.3 Prepare the Ab-FITC working solution

7.6.3.1 Indicate which marker is being tested, record the lot number and release date for the qualified Ab-FITC conjugate being used, and record the recommended dilution of Ab-FITC conjugate stock to use for the assay in the Batch Record (Appendix 1, Section 2A).

- The laboratory is responsible for maintaining copies of the Batch Records for all qualified batches of Ab-FITC conjugate in use (e.g., SOP LHTP003.8.1.1 for qualification of γ H2AX-AF488).
- Generally, Ab-FITC stock solutions should be used within 2 y of date of receipt. It is suggested to test antibody quality every 6 months and maintain copies of Batch Records from all validation runs.

7.6.3.2 Record the lot number and expiration date for the Bond Primary Antibody Diluent in the Batch Record (Appendix 1, Section 2A).

7.6.3.3 Using the calculations in Appendix 1, Section 2A, determine the total volume of working solution needed for all samples and volume of Ab-FITC needed to make the working solution.

7.6.3.4 Prepare the Ab-FITC working solution in Bond Primary Antibody Diluent using the calculations in Appendix 1, Section 2B. Pipette the calculated volumes of Ab-FITC stock solution and Bond Primary Antibody Diluent into a 1.5-mL Sarstedt screw cap tube and gently pipette up and down to mix. Cap and set aside with the CTC Kit.

7.6.3.5 The antibody working solution should be discarded at the end of every day. If more than one assay run is being performed in a day, the residual antibody volume from the previous run can be used to make additional antibody solution for the next run, but this should be noted as a deviation in the Batch Record (Appendix 1, Section 3).

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7.6.4 Running a Batch

- 7.6.4.1 On Main Menu, click on **Run Batch** and press **Enter**. Enter user password to log into the AutoPrep. If you have not run the Daily Cleaning protocol in the past 72 h, it will need to be done prior to running clinical samples (see Appendix 2, Section 1 for details).
- 7.6.4.2 Enter the following information for **Batch Setup**, pressing **Enter** after each. Information for the batch will be updated in the upper right-hand corner of the screen as information is entered.

Field	Fill in
Select Reagent Kit	<i>CellSearch CTC kit</i>
Select Marker Test	<i>User Defined Assay</i> Example: “ <i>gH2AX</i> ” or “ <i>p16INK4</i> ”
Select Control	Select <i>Yes</i> or <i>No</i> to indicate if this batch contains a control sample
Select Total number of Samples	Select the <i>batch size</i> (e.g., for 1 control + 7 samples, select 8)

7.6.5 Loading AutoPrep

- 7.6.5.1 The screen will prompt you to “**Load Reagent Kit**” with a reminder to load the antibody tube. Remove cap from the 1.5-mL Ab-FITC working solution tube and place it into position 1 of the reagent carousel and then press **Enter**.
- 7.6.5.2 The screen will again prompt you to “**Load Reagent Kit.**” Remove caps from each bottle of the CTC Kit and load the kit onto the reagent carousel. Place carousel in the AutoPrep and then press **Enter**.
- Save the caps from the CTC Kit bottles to recap bottles for storage after the run is completed; caps preserve liquid volume better than the provided evaporation cover.
- 7.6.5.3 Prepare one MagNest for each sample to be processed by placing a cartridge (supplied with CTC Kit) into each MagNest and then placing the loaded MagNest into the AutoPrep. Press **Enter** after each MagNest is loaded; the cartridge ID will appear on the screen as it is scanned.
- 7.6.5.4 Load samples into the AutoPrep following the on-screen instructions, with bar codes facing forward, in the order recorded in the Sample Information Table (Appendix 1, Section 1). If a control sample is being run, it should be the first tube loaded.
- If a new Control Kit lot number is detected by the system, you will be requested to scan the barcode from the Control Kit **box** and follow the on-screen instructions.

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7.6.5.5 After each sample is loaded, press **Enter**. The screen will prompt “**Run Marker Test?**”

- For the control sample, arrow down and select **NO**.
- For the clinical samples, press **Enter** to select **YES** – this will ensure the Ab-FITC solution is run with the samples.
- Once the run starts, the marker status cannot be changed.

7.6.5.6 Once all samples are loaded, select **Start** and press **Enter** to start the run. The predicted completion time for each sample will be listed under the Time Ready column on the **Processing Samples** screen. The AutoPrep run should begin **within 1 h** of sample preparation (< 97 h after blood draw time).

7.6.5.7 Once the run has started, update the clinical sample information as follows. Select **Edit**, press **Enter**, select **Edit sample information**, and press **Enter** again. Use the arrows keys to select the sample to be edited and press **Enter**. Once edited, select **Save**.

- Sample information can be edited any time prior to the completion of a sample run.
- Enter the Patient/Sample ID from the Sample Information Table (Appendix 1, Section 1). Due to character limitations, the ID can be abbreviated by entering the CTEP#/Clinical Protocol# in the Comments field.
- The blood collection date and time should be entered into the Draw Date and Draw Time fields
- In Comments field record the following:
 - o CTEP# and/or Clinical Protocol#
 - o Collection location (e.g., NCI CC)
 - o Deviations and notes for specific samples
- If any of the above information is recorded in the Batch Record (Appendix 1, Section 3) instead of on the CellTracks, be sure to identify which Patient/Sample ID the information pertains to in the Batch Record.

7.7 Unloading Samples From AutoPrep

Note: Samples can be unloaded after the entire batch is complete or as each sample is finished processing.

7.7.1 Once a sample is completed, the Status column updates to “Complete,” and the MagNest door will open for a brief period of time to allow removal of the MagNest. If this time window is missed, the MagNest for that sample can only be removed once the batch is completed; other MagNests can be removed as they are completed.

7.7.2 Upon batch completion, the system will display an **Unload** button. Press **Enter** and follow the on-screen prompts to remove the remaining MagNests.

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- 7.7.3 Remove MagNests from the AutoPrep and immediately cap them, tapping the side of the MagNest to minimize bubbles. Lay each capped MagNest flat, in the dark (e.g., in a drawer), and wait 20 min (< 24 h) before starting analysis (SOP Step 7.8).
- 7.7.4 Unload the 15-mL AutoPrep tubes and discard in appropriate biohazardous waste container.
- 7.7.5 Unload the reagent carousel and discard any unused Ab-FITC working solution. If there are remaining solutions in the CTC Kit, recap all bottles, return to the CTC Kit box, and store at 2°C to 8°C until next use.
- 7.7.6 If this was the last AutoPrep run of the day, run the Daily Cleaning protocol (Appendix 2, Section 1).

7.8 CellTracks Analyzer II Image Acquisition

- 7.8.1 Turn the Analyzer on and then the computer. The Analyzer software will boot automatically. When prompted, enter the user password to log into the Analyzer. **Note:** One sample (MagNest) will be processed at a time in the Analyzer.
 - 7.8.1.1 The Home Screen has tabs across the top, called folders, which when selected display additional subfolders. The buttons across the bottom of the Home Screen are for the tool bar.
- 7.8.2 Select the Lamp button on the tool bar and click **YES** in the dialog box to turn on the lamp. Wait at least 15 min for the lamp to warm up. An indicator will flash green during warm up and will remain steady once the lamp is ready.
- 7.8.3 If a control sample has not yet passed QC for the day, analyze the control sample first by following the protocol in SOP Section 8.0.
- 7.8.4 To analyze a clinical sample, select the **Patient Data** folder on the Home Screen and then the **Sample Data** subfolder.
 - 7.8.4.1 The Analyzer will prompt you if **System Verification** is required (see Appendix 3 for details).
- 7.8.5 Once a clinical sample has sat in the dark for at least 20 min (< 24 h), take the MagNest out, wipe the cover slip of the cartridge with lens paper, and then insert it into the Analyzer.
- 7.8.6 The sample data stored on the data button of the MagNest from the AutoPrep will be uploaded into the Analyzer. Visually verify that the sample information is correct. Click the **Edit** button and change the ***Test Protocol** field to **Research**. Correct any errors and add comments as needed. Select **Save** to continue.
- 7.8.7 Click the **Start** button to begin a scan.

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7.8.8 Confirm edge detection.

7.8.8.1 If the “# of Frames” is less than 175 (bottom left-hand corner of screen) or if the green line in any of the images does not line up with the image edges, the cartridge may not be inserted properly – select the **Cancel** button, remove and reinsert the MagNest, and restart the scan.

If the green lines still do not line up with the image edges, first click **OK** to clear the dialog box, and then click on the image. Line up the green line with the image edges by making fine movements using the Arrow keys, medium movements using the Shift key + Arrow keys, and coarse movements using the Ctrl key + Arrow keys.

Click **Enter** in the dialog box to confirm the changes and then the **Accept** button to continue.

7.8.8.2 If the edges of the cartridge are detected correctly, click **OK** to clear the dialog box, and then the **Accept** button to continue.

7.8.9 Confirm auto focus by clicking **OK** to clear the dialog box and then clicking the **Accept** button to continue.

7.8.10 The Analyzer will scan the cartridge. Image acquisition will take approximately 10 min for all 4 filters. A scan can be cancelled at any time by clicking the **Cancel** button; however, all of the images collected will be deleted.

7.8.11 A pop-up window will state **Scan complete**; click **OK** to continue. Open the sample door and remove the MagNest. The next MagNest can be inserted and scanned immediately. Return to SOP Step 7.8.4 to scan additional clinical samples.

7.8.12 If this is the last scan of the day, select the **Lamp** button on the tool bar and click **YES** in the dialog box to turn off the lamp.

7.9 Review and finalize the Batch Record (Appendix 1) and obtain required signatures. Document ANY and ALL deviations from this SOP in the Batch Record (Appendix 1, Section 3) and/or in the comments field of the CellTracks Analyzer for a specific patient sample.

7.10 Proceed to SOP Section 9.0 to analyze clinical samples.

8.0 CONTROL SAMPLE ANALYSIS AND QUALITY CONTROL

A control sample from the Control Kit checks the overall CellSearch System performance. One control sample should be run on each day of clinical sample testing and each time a new CTC Kit lot is used.

8.1 CellTracks Analyzer II Image Acquisition

8.1.1 Select the **QC Test** folder and then the **Control Data** subfolder.

8.1.2 Once the control sample has sat in the dark for at least 20 min, take the MagNest out, wipe the cover slip of the cartridge with lens paper, and insert it into the Analyzer.

8.1.3 The control sample data, including expected low and high control ranges, are stored on the data button of the MagNest from the AutoPrep and will be uploaded to the Analyzer. Visually verify that the Control Kit information is correct. Use the **Edit** button to correct errors or add comments and select **Save** to continue.

8.1.4 Click the **Start** button to begin a scan.

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8.1.5 Confirm edge detection.

8.1.5.1 If the “# of Frames” is less than 175 (bottom left-hand corner of screen) or if the green line in any of the images does not line up with the image edges, the cartridge may not be inserted properly – select the **Cancel** button, remove and reinsert the MagNest, and restart the scan.

If the green lines still do not line up with the image edges, first click **OK** to clear the dialog box, and then click on the image. To line up the image edges with the green line, make fine movements using the Arrow keys, medium movements using the Shift key + Arrow keys, and coarse movements using the Ctrl key + Arrow keys.

Click **Enter** in the dialog box to confirm the changes and then the **Accept** button to continue.

8.1.5.2 If the edges of the cartridge are detected correctly, click **OK** to clear the dialog box, and then the **Accept** button to continue.

8.1.6 Confirm auto focus by clicking **OK** to clear the dialog box, and then clicking the **Accept** button to continue.

8.1.7 The Analyzer will scan the cartridge. A pop-up window will state **Scan complete**; click **OK** to continue. Open the sample door and remove the MagNest.

8.2 Identification of Control CTCs

8.2.1 Select the **QC Data** folder and then the **Control** subfolder. Click on the row matching the control sample just processed and then select the **Review** subfolder. Sample details for the row selected will be displayed at the bottom of the **Control** subfolder screen.

8.2.2 Select a cell image in the image gallery to classify as a high or low control cell. The Control Kit sample contains both high (~1000 cells) and low (~50 cells) control cells; each cell population is labeled with a different fluorescent membrane dye for identification.

8.2.3 Most of the high control cells will be automatically assigned by the Analyzer, but some high controls will be presented in the image gallery for user review. Click on the images in the gallery and classify them as high or low control cells. The resulting counts must fall within the expected ranges for high and low controls as recorded from the barcode label affixed to the Control Kit box (see SOP Step 8.3).

8.2.3.1 For images containing CK-PE+/DAPI+/LOW-/HIGH+ cells, classify it as a high control cell by clicking on the HIGH image.

8.2.3.2 For images containing CK-PE+/DAPI+/LOW+/HIGH- cells, classify it as a low control cell by clicking on the LOW image.

8.2.3.3 For images containing both high and low control cells in the same frame, classify it as a low control cell by clicking on the LOW image.

8.2.4 Once all high and low control cells have been assigned, click **Done** on the right-hand side of the screen.

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8.3 Control Sample Quality Control (QC)

- 8.3.1 Next, select the **QC Data** folder and then the **Control Table** subfolder. Select the row matching the control sample just analyzed.
- 8.3.2 Select the **Report** subfolder and verify that the high and low control counts in the Results section are within defined Control Kit ranges. The **Status** on the **Control Report** will be assigned as either **Pass** or **Fail** for the control kit.
- 8.3.3 If the control sample passes QC, write “Pass” in the Collection Location column of the Sample Information Table for the control sample and place your initials at the top of the table as indicated in the Batch Record (Appendix 1, Section 1).
- 8.3.4 If the control sample fails QC, run a second control sample through the AutoPrep and Analyzer. If the second control sample fails QC, any patient samples run with the initial control sample should be discarded, and Veridex technical support should be contacted.

- 8.4 Once the control sample passes QC, return to SOP Step 7.8.4 to start Image Acquisition for clinical samples.

9.0 CLINICAL SAMPLE ANALYSIS

9.1 Identification of CTCs and Ab-FITC–Positive CTCs

- 9.1.1 Select the **Patient Data** folder and then the **Sample Table** subfolder. Click on the row matching the clinical sample to be analyzed and then select the **Review** subfolder. Sample details for the row selected will be displayed at the bottom of the **Sample Table** subfolder screen.
- 9.1.2 A gallery of acquired cell images for that sample will be displayed. Page down to view all images. Cell type assignments are performed manually. Columns that are marked with the Immunicon logo may be selected.
- 9.1.3 Click on cell images in the DAPI/CK-PE column to classify them as CTCs. CTCs are DAPI+, CK-PE+, FITC-, and CD45/APC-. Detailed criteria are discussed during the Veridex training course.
- 9.1.4 Click on cell images in the FITC column to classify them as Ab-FITC–positive CTCs. These should be DAPI+, CK-PE+, FITC+, and CD45/APC-.
- 9.1.5 Click **Done** after all images have been reviewed.

9.2 Generate Sample Reports

- 9.2.1 Select the **Patient Data** folder and then the **Sample Table** subfolder. Click on the row to select a sample and then select the **Report** subfolder.
- 9.2.2 A preview of the report appears on the screen. A report can only be printed if **Done** was selected after clinical sample analysis (SOP Step 9.1.5). Appendix 4 has an example CellTracks Research Report with the field data entered in SOP Step 7.6.5.6 outlined.
- 9.2.3 Once all patient samples have been processed, print **two (2) copies** of the CellTracks Research Report for each patient. Attach one copy of each patient report to the Batch Record. The second copy will be sent to the Clinical PI as the Clinical Data Report for that patient. **Note:** If digital copies of the CellTracks Research Reports are sent to the PI, only one copy will need to be printed.

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9.2.4 The CellTracks Analyzer automatically records the Cell Tracks Start Date and Time. This should be < 97 h after the time of blood collection (maximum 96 h in CellSearch tube + ~1 h prep time). If this is > 97 h, a deviation should be noted on the Research Report and/or in the Batch Record (Appendix 1, Section 3).

9.3 A second certified Veridex user should review the Batch Record and sample reports and sign the Batch Record affirming the data contained within the reports are correct (Appendix 1, Section 4).

9.4 If there are any deviations to the SOP, the Laboratory Director/Supervisor should review the Batch Record with attached Research Reports and sign the Batch Record affirming the data contained for **each** patient sample are correct (Appendix 1, Section 5).

10.0 ARCHIVING DATA

Due to the large amount of data collected with each analysis, data will need to be archived at regular intervals. The **Archive** function is used to move data and images off of the Analyzer and onto DVD+Rs for long-term storage. Archive samples only after image **Review** is complete (SOP Step 9.1.5).

10.1 Use only high-quality DVD+Rs (Section 6.0) to ensure data integrity.

10.2 Depending on the number of images associated with each sample, data from several samples can be stored on a DVD+R. It is recommended to store data in numerical order by specimen ID.

10.3 Select the **Archive** folder. You can select specific samples to archive together, or archive in numerical order. Press the CTRL key on the keyboard and click the samples that you want to archive together.

10.4 The system will display a dialog box stating the number of samples being archived and will assign a disk number. Insert a blank DVD+R and click the **Start** button.

10.5 Label the DVD+R with the archive (disk) number and sample information. Record the archive number for each sample in the Sample Information Table.

10.6 Archived data will no longer be stored in the Analyzer. Archived samples will be identified in the **Patient Data** folder under the **Archived Disk** column.

10.7 Maintain the DVD+Rs in a local storage location until the clinical trial is completed, then move the DVD+Rs to a secure storage location.

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APPENDIX 1: BATCH RECORD

NOTE: Record times using **military time** (24-h designation); e.g., specify 16:15 to indicate 4:15 PM.

NOTE: If any portion of the Batch Record is recorded in a digital copy, cross through that section, state "See attached sheet," and initial. A copy of the completed, digital table must be printed and attached to the Batch Record in order to maintain a complete audit trail.

Certified Assay Operator: _____

DCTD certification number: _____

Facility/Laboratory Running SOP: _____

1. Sample Information Table

Has a control sample passed QC today? ☐ Yes ☐ No

If No, run a control sample with this assay (tube #1). **Initial Here** _____, once QC is passed. If control sample fails, the entire assay run fails and should not be used to report clinical sample results.

Tube Order*	Patient/Sample ID
<i>Ex.</i>	<i>1234-001026-400</i>
<i>Ex.</i>	<i>5678-001035-C1D1</i>
1	
2	
3	
4	
5	
6	
7	
8	

* The last sample in the first sample run each day should be a CTC control sample.

BATCH RECORD: INITIALS _____ DATE: _____

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2. Preparation of Ab-FITC Working Solution (SOP Step 7.6.3)

A. Ab-FITC Working Solution Calculations

Primary antibody in Ab-FITC conjugate: ☐ γ H2AX ☐ p16^{INK4a} ☐ _____

Ab-FITC conjugate Lot #: _____

Qualification Date: _____ / _____ / _____

Recommended *DILUTION* for *Ab-FITC Stock*: _____ 1 : _____

Bond Primary Antibody Lot #: _____

Diluent Expiration Date: _____ / _____ / _____

a. **Total Vol.** Calculation (μ L)

Total number of samples to be processed:

_____ x 150 μ L/sample = _____ μ L

Plus 300 μ L residual volume + _____ 300 μ L

Total Vol. needed for staining = _____ μ L

b. *Vol. Ab-FITC Stock* Calculation (μ L)

$\frac{\text{Total Vol. } (\mu\text{L})}{\text{Recommended } DILUTION \text{ of Ab-FITC Stock}} = \underline{\text{XX.X}} \mu\text{L (Vol. Ab-FITC Stock)}$

$\frac{\text{_____ } \mu\text{L}}{\text{(DILUTION factor)}} = \text{_____ } \mu\text{L (Vol. Ab-FITC Stock)}$

B. Ab-FITC Working Solution Preparation

_____ μ L Vol. Bond Primary Antibody Diluent
(Total Vol. – Vol. Ab-FITC Stock)
 _____ μ L Vol. Ab-FITC Stock

BATCH RECORD: INITIALS _____ DATE: _____

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3. Notes, including any deviations from the SOP:

Deviations can also be recorded in the comments section of the AutoPrep or Analyzer for the individual patient sample.

4. Secondary Review of Clinical Samples

Certified Assay Operator: _____ (PRINT)

_____ (SIGN)

Date: ____ / ____ / ____

5. Laboratory Director/Supervisor Review of Batch Record and CellTracks Research Reports

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: ____ / ____ / ____

BATCH RECORD: INITIALS _____ DATE: _____

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BATCH RECORD: INITIALS _____ DATE: _____

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APPENDIX 2: CELLSEARCH SYSTEM MAINTENANCE

1. Daily

The AutoPrep will prompt you to run a Daily Cleaning if the machine was just turned on for the first time of the day, or if it has been > 72 h (3 days) since the last Daily Cleaning.

- A. On the Main Menu click on **Daily Cleaning** and press **Enter**.
- B. Prepare and attach the following solution bottles:
 - a. Waste bottle – empty and then fill with 400 mL DI water
 - Empty in appropriate biohazardous waste container
 - b. Cleaning solution bottle – fill with 0.26% bleach, diluted in DI water
 - c. Instrument buffer bottle – remove and replace with full DI water bottle
- C. Press **Enter** to Start Daily Cleaning (takes about 1 h).
- D. Once completed, prepare and attach the following solution bottles:
 - a. Waste bottle – empty and then fill with 400 mL bleach, full strength
 - Empty in appropriate biohazardous waste container
 - b. Cleaning solution bottle – fill with 0.26% bleach, diluted in DI water
 - c. DI water bottle – remove and replace with full instrument buffer bottle

Remember to switch the instrument buffer bottle back to buffer before running samples. Refer to the CellTracks AutoPrep User's Guide for additional details.

2. Weekly and Monthly Maintenance Cleaning

Follow the recommended CellTracks AutoPrep weekly and monthly maintenance cleaning schedule; refer to the User's Guide for detailed instructions. It is recommended to maintain a maintenance log with the CellTracks AutoPrep System to ensure cleaning is being completed as specified and on schedule.

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APPENDIX 3: DAILY SYSTEM VERIFICATION

1. System Verification

The CellTracks Analyzer II will prompt you to run a System Verification before scanning the first sample if the machine was just turned on for the first time of the day, if the software was restarted, or if it has been 10 h since the last System Verification.

- A. Select the **QC Test** folder and then select the **Verification** subfolder.
- B. Prepare a system verification cartridge and its associated MagNest.
 - a. Be sure it is before the expiration date of the cartridge.
 - b. Wipe the cover slip of the cartridge with lens paper and load it into the Analyzer.
 - c. If a new verification cartridge is being used, click **edit verification settings**, enter the new cartridge ID number, and then click **OK**.
- C. Click the **Start** button in the system verification box located at the bottom of the screen.
- D. Remove the system verification cartridge from the instrument when the procedure is complete.
- E. Select the **QC Data** folder and then select the **Verification** subfolder to access the verification list.
- F. Verify that the system verification Result was Pass. If system verification fails, Veridex technical support should be contacted.

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APPENDIX 4: EXAMPLE CELLTRACKS RESEARCH REPORT

Red boxes indicate patient and sample data entered into the CellTracks AutoPrep in SOP Step 7.6.5.7.



RESEARCH REPORT

Facility: NCI-Frederick

Report Date: 03/29/2013 09:18

Sample ID: 001025-C1D1
Volume: 5.0 mL

Patient ID: 001025

Cartridge ID: 01125195
Scan #: 1

Instruments and Operators

CellTracks Analyzer II®

Serial #: CT0909059
Test Protocol: CTC Research
Scan Operator ID: SAK
Scan Date/Time: 03/28/2013 13:49
First Reviewer ID: LHW
Review Date/Time: 03/28/2013 15:36
Last Reviewer ID: SAK
Review Date/Time: 03/29/2013 09:18

CellTracks® AutoPrep® System

Serial #: AP0706012
Operator ID: SAK
Prep Date: 03/28/2013
Prep Time: 12:59
Sample Position: 3

Draw Date: 03/26/2013
Draw Time: 09:26

Batch Information

Reagent Kit

Kit ID: CellSearch® CTC
Kit Lot: 0162A
Expiration: 10/01/2013

Marker Reagent

Marker ID: gH2AX-AF488
Marker Lot:
Expiration:

Results

For Research Use Only. Not for use in diagnostic procedures.

Result	# Cells	% of Cells
CTC+:	8	100.00
CTC+/gH2AX-AF488+:	3	37.50
CTC+/gH2AX-AF488-:	5	62.50
Unassigned:	107	

Comments

CTEP#: 1234
NCI Clinical Center
Sample contained 5 mL blood

Report Authorization: _____

LW

Date: _____

3/29/2013