**TRAINING UPDATE**

| | | | |
|---|---|---|---|
| **Lab Location:** | SGMC and WAH | **Date Implemented:** | 10.27.2015 |
| **Department:** | Blood Bank | **Due Date:** | 11.15.2015 |

**DESCRIPTION OF PROCEDURE REVISION**

| **Name of procedure:** |
|---|
| Antibody Screen, Manual Capture |
| **Description of change(s):** |
| If the internal positive control in the RS-3 strip fails for any reason, the tech **MUST** write a PI/variance report. |

## Electronic Document Control System


**Document No.:** WAH.BB115[2]
**Title:** Antibody Screen, Manual Capture
**Owner:** LESLIE BARRETT
**Status** INWORKS
**Effective Date:** 22-Nov-2015 **Next Review Date:**

Technical SOP

| | |
|---|---|
| **Title** | **Antibody Screen, Manual Capture** |
| **Prepared by** | Stephanie Codina Date: 10.20.2011 |
| **Owner** | Stephanie Codina Date: 10.20.2011 |

| **Laboratory Approval** | **Local Effective Date:** | |
|---|---|---|
| **Print Name and Title** | **Signature** | **Date** |
| *Refer to the electronic signature page for approval and approval dates.* | | |
| | | |
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| **Review** | | |
|---|---|---|
| **Print Name** | **Signature** | **Date** |
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Form revised 10/31/02

## TABLE OF CONTENTS



## 1. TEST INFORMATION

| Assay | Method/Instrument | Local Code |
|---|---|---|
| Antibody Screen | Manual Capture | TS or AS |

| Synonyms/Abbreviations |
|---|
| Indirect Antiglobulin Test, Indirect Coombs, Screen |

| Department |
|---|
| Blood Bank |

## 2. ANALYTICAL PRINCIPLE

Capture-R Ready Screen is a modified solid phase antibody detection system based on the procedures of Plapp et al and Juji et al. Membranes of red cells have been bound to and dried on the surfaces of polystyrene micro wells. The membrane antigens are used to capture red cell specific antibodies from patient or donor plasma. Following a brief incubation period, unbound residual immunoglobulins are rinsed from the wells and replaced with a suspension of anti-IgG-coated indicator red cells. Centrifugation brings the indicator red cells in contact with antibodies bound to the reagent red cell membranes. In the case of a positive test, the migration of the indicator red cells to the bottom of the well is impeded as anti-IgG –IgG complexes are formed on the surface of the immobilized reagent layer. As a consequence of antibody bridging, the indicator cells adhere to the screening cells as a second immobilized layer. In the absence of detectable antigen-antibody interactions (negative test), the indicator red cells will not be impeded during their migration and will pellet to the bottom of the wells as tightly agglutinated red cell buttons.

## 3. SPECIMEN REQUIREMENTS

### 3.1 Patient Preparation

| Component | Special Notations |
|---|---|
| **Fasting/Special Diets** | N/A |
| **Specimen Collection and/or Timing** | N/A |
| **Special Collection Procedures** | N/A |
| **Labeling** | Patient identification must be confirmed and blood bank armband system utilized. Refer to procedure "Sample Specifications for Blood Bank Testing" for details. |

### 3.2 Specimen Type & Handling

| Criteria | |
|---|---|
| **Type -Preferred** | Plasma ($K_3$ EDTA, $K_2$ EDTA), Whole Blood ($K_3$ EDTA, $K_2$ EDTA) |
| **-Other Acceptable** | None |
| **Collection Container** | Lavender top tube, Pink top tube |
| **Volume - Optimum** | 10ml of whole blood or 5ml of plasma |
| **- Minimum** | 4 ml of whole blood or 2ml of plasma |
| **Other Considerations** | The specimen will need to be labeled with a blood bank labeling system if used for possible transfusion |
| **Transport Container and Temperature** | Same as above, at room temperature |

| Criteria | |
|---|---|
| **Stability & Storage Requirements** | Room Temperature: 24 hours<br>Refrigerated (1-10°C): 7 days<br>Frozen (≤ -20°C): 12 months (unacceptable for whole blood) |
| **Timing Considerations** | Test as soon as possible following collection |
| **Unacceptable Specimens & Actions to Take** | 1) Specimens that are not properly labeled.<br>2) Specimens with any anticoagulant other than EDTA.<br>3) Whole blood in serum separator tube (SST).<br>4) Grossly hemolyzed, lipemic, and/or icteric specimens.<br>5) Clotted specimens.<br>6) Frozen whole blood.<br>Reject specimen. Refer to procedure "Sample Specifications for Blood Bank Testing" for details on recollection and documentation. |
| **Compromising Physical Characteristics** | **Condition** / **Slight** / **Moderate** / **Marked**<br>**Hemolysis** / OK / Unacceptable / Unacceptable<br>**Icterus** / OK / OK / Unacceptable<br>**Lipemia** / OK / OK / Unacceptable |
| **Other Considerations** | None |

## 4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

### 4.1 Reagent Summary

| Reagents/Kits | Supplier & Catalog Number |
|---|---|
| Capture-R Ready Screen (3) Plates | Immucor 66813 or equivalent, stockclerk 134185 |
| Capture LISS | Immucor 6420 or equivalent, stockclerk 118835 |
| Capture-R Indicator Cells | Immucor 6428 or equivalent, stockclerk 141692 |
| pHix Buffer Solution | Immucor 5070 or equivalent, stockclerk 152340 |
| Isotonic Saline, Certified blood bank saline | Stock Clerk # 9015482 |

### 4.2 Reagent Preparation and Storage

**NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.**

**Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.**

| | |
|---|---|
| **Reagent** | Capture-R Ready Screen (3) Plates |
| **Container** | 1 x 8 strips of wells carrying the bound and dried red cell membranes of three different group O donors. Twelve 1 x 8 strips are packaged with a support frame and enclosed in a foil pouch to which a dessicant and moisture indicator have been added. |
| **Storage** | 1-30°C |
| **Stability** | Strips in unopened packages may be used until the expiration date on the package provided the humidity indicator enclosed within the pouch does not show presence of moisture (by humidity indicator turning from blue to pink). Strips removed from pouches should be used within eight hours. |
| **Preparation** | Ready to use |

| | |
|---|---|
| **Reagent** | Capture LISS |
| **Container** | 11.5 mL vial |
| **Storage** | 1-10°C |
| **Stability** | Stable until manufacturer's expiration date. |
| **Preparation** | Ready to use as supplied. |

| | |
|---|---|
| **Reagent** | Capture-R Indicator Cells |
| **Container** | 11.5ml each |
| **Storage** | 1-10°C |
| **Stability** | Stable until manufacturer's expiration date. |
| **Preparation** | Resuspend red cells before use by gently inverting each vial several times. |

| | |
|---|---|
| **Reagent** | pHix |
| **Container** | 200 mL bottle |
| **Storage** | 18-30°C |
| **Stability** | Stable until expiration date on vial |
| **Preparation** | Ready to use. Concentrate is added to saline to create PBS. |

| | |
|---|---|
| **Reagent** | Isotonic Saline |
| **Container** | 20L or 10L container |
| **Storage** | 18-30°C |

| | |
|---|---|
| **Stability** | Stable until expiration date on container until opened. Stable for 30 days once opened and after pHix is added. |
| **Preparation** | pHix is added prior to use. |

## 5. CALIBRATORS/STANDARDS

N/A

## 6. QUALITY CONTROL

### 6.1 Controls Used

| Controls | Supplier and Catalog Number |
|---|---|
| Capture-R Positive Control Serum (Weak) and Capture-R Negative Control Serum | Immucor 66245 or equivalent, stock clerk 134184 |

### 6.2 Control Preparation and Storage

**NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.**

| | |
|---|---|
| **Reagent** | Capture-R Positive Control Serum (Weak) and Capture-R Negative Control Serum |
| **Container** | 5.0 mL vials |
| **Storage** | 1-10°C |
| **Stability** | Stable until expiration date on vial |
| **Preparation** | Ready for use as supplied |

### 6.3 Frequency

The positive and negative control sera will be tested on each day of use.

The Capture-R Ready Screen also has an internal, positive control well to verify that the indicator cells were not neutralized during the assay process. The positive control well is coated with an IgG anti-D sensitized red blood cell membrane in the manufacturing process.

### 6.4 Tolerance Limits

Control material must perform as expected (see package insert for acceptability of reactions). Continued failure of the controls to perform properly on repeated testing may indicate that one or more of the Capture-R reagents have deteriorated or that tests

are consistently being performed incorrectly. Any QC that deviates from expected results found on the daily QC sheet must be investigated and corrected prior to release of patient results. Refer to procedure 'Quality Control Failure Resolution'.

### 6.5 Review Patient Data

Review patient results for unusual patterns, trends, or distributions in patient results such as an unusually high percentage of abnormal results. Notify a supervisor if these circumstances exist.

### 6.6 Documentation

Quality control results are documented on the quality control form.

### 6.7 Quality Assurance Program

Participation in CAP proficiency testing.

## 7. EQUIPMENT and SUPPLIES

### 7.1 Assay Platform

N/A

### 7.2 Equipment

CSW 100 Capture Strip Well Washer
Incubator P2 (37 ± 1°C)
Immuspin (centrifuge)
Illuminated surface
Timer

### 7.3 Supplies

Disposable pipettes
Blank strips of wells for balance in centrifuge

## 8. PROCEDURE

**NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.**

**The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included in the appropriate notebook/file.**

| Step | Action |
|---|---|
| 1 | Bring the Capture reagents to room temperature before use (18-30°C). |
| 2 | Confirm specimen acceptability and specimen labeling per procedure, "Sample Specifications for Blood Bank Testing." |
| 3 | Perform a history check per procedure, "Patient History Check." |
| 4 | Centrifuge the whole blood specimen for 5-10 minutes at 3000-3600 rpm to separate plasma from cells. |
| 5 | Remove the required number of Capture-R Ready Screen strips from the protective pouch and place in the frame holder. Return all unused strips, desiccant, and humidity indicator to pout and reseal.<br>A. The strip wells are sealed in a foil pouch with a desiccant and humidity indicator. The strip wells should **not** be used if the humidity indicator shows the presence of moisture by turning from blue to pink.<br>B. The humidity indicator is acceptable if the color of the circle is as blue or bluer than the rectangle.<br>  <br>C. The humidity indicator is **un**acceptable if the color of the circle is lighter blue than the rectangle or pink in color.<br>  |

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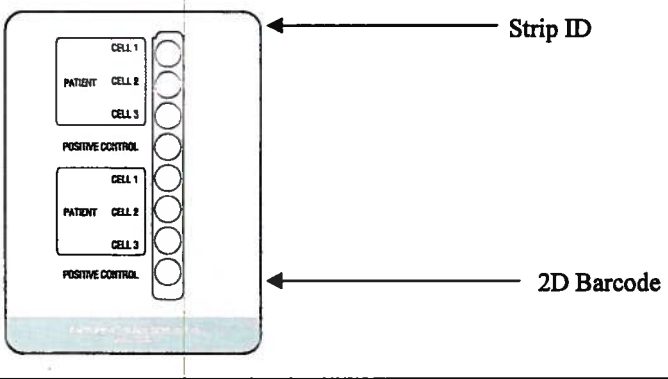
| Step | Action |
|---|---|
| 6 | Check the bottom of the strip. Do not use the strip if it is not imprinted to show the test identification. The arrangement of the Capture-R Ready Screen strip is:   |
| 7 | Place the strip in a frame holder so that the numbers are readable. The 2D barcode will be on the side closest to you. |
| 8 | Label each strip with the patient or control identifiers.<br>A. Label the tab of the strip closest to the wells in which the specimen will be placed.<br>B. For patients, use the first and last initial or the first 3 letters of the last name. Use additional identifiers if needed to differentiate between specimens.<br>C. For controls, write "POS" for positive control and "NEG" for negative control. |
| 9 | Add two drops (100 ± 10 µL) of Capture LISS to each test well. The LISS will be purple when added to an empty test well. |
| 10 | Add 1 drop (50 ± 5 µL) of test serum or plasma to the corresponding test wells.<br>A. Add 1 drop of patient plasma to each appropriately-labeled well.<br>B. Add 1 drop of positive control serum to each well labeled "POS."<br>C. Add 1 drop of negative control serum to each well labeled "NEG."<br>D. The LISS turns blue in the presence of plasma protein. Retention of a purple color may indicate that the test plasma was inadvertently omitted from the well. |
| 11 | Tap the plate gently to mix and dislodge any bubbles.<br>A. If bubbles remain, try to "pop" by further gently tapping.<br>B. If this is not successful, carefully use a wooden stick to pop the bubble, being careful not to touch or disturb the monolayer. |
| 12 | Incubate the strips in the Immucor Incubator P2 at 37 ± 1°C for no less than 20 minutes and no longer than 60 minutes. |

| Step | Action |
|---|---|
| 13 | Wash the strips following incubation per one of the following procedures,<br>A. Immucor CSW 100 Capture Strip Well Washer (preferred)<br>B. Manual Wash Techniques (optional) |
| 14 | Add 1 drop (50 ± 5 µL) of well-mixed Capture-R Indicator Cells to each of the wells.<br>A. Dispense this reagent by using the dropper at a 45° angle.<br>B. Avoid touching the tip of the dropper. Contamination can neutralize the AHG component. |
| 15 | Immediately centrifuge the strips for 2 minutes at 530 rcf. |
| 16 | Place the strip on an illuminated surface and examine for the presence or absence of Indicator Red Cell adherence. Grade reactions and record results directly into the LIS.<br>A. If the positive control is not positive, test results are invalid and must be repeated. If the positive control fails for any reason, document the failure on a PI/variance form and provide to the Technical Supervisor for review.<br>B. Wells can be saved and reread manually for up to 48 hours following testing.<br>a. Cover the wells to prevent evaporation.<br>b. Store the wells in the refrigerator at 1-10°C. |
| 17 | If the interpretation is "positive."<br>A. Call the patient care area to inform them that the antibody screen is positive and there will be a delay in providing crossmatch-compatible blood products.<br>B. Document the phone call in the LIS.<br>a. Access Sunquest function, "Blood Order Processing."<br>b. In the "Add Spec Test" field, press the "Shift" button and type "W" or type ";BBCALL" to add the "Called to" field.<br>c. Freetext who you called and at what time. For example, "Positive antibody screen called to J. Doe, RN at 1400."<br>d. Press the "Save" button.<br>C. Perform antibody identification per procedure.<br><br>Note: Blood products should not be released until antibody identification is complete. A pathologist should be consulted if blood is needed before the antibody identification is complete. |

## 9. CALCULATIONS

N/A

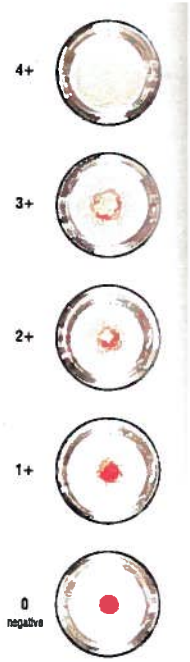
## 10. REPORTING RESULTS AND REPEAT CRITERIA

### 10.1 Interpretation and Repeat Criteria

Negative Result –the button of Capture-R Indicator Cells are at the bottom of the well with no area of adherence..

Positive Result –the Capture-R Indicator Cells are adhered to part of or the entire reaction surface.

**Reaction Grading Guide**




### 10.2 Rounding

N/A

### 10.3 Units of Measure

N/A

### 10.4 Clinically Reportable Range (CRR)

N/A

### 10.5 Repeat Criteria and Resulting

10.5.1 If the correct reaction is not obtained for the positive control, test reactions are invalid and cannot be reported. Document the failure of the positive control on a PI/variance form and provide to the Technical Supervisor for review.

10.5.2 If the antibody screen is negative, report as negative. If the antibody screen is positive, report as positive and perform antibody identification.

## 11. EXPECTED VALUES

### 11.1 Reference Ranges

Negative

### 11.2 Critical Values

None established

### 11.3 Priority 3 Limit(s)

Positive antibody screens are called to the patient care area when it will take ≥ 2 hours to provide compatible blood products.

## 12. CLINICAL SIGNIFICANCE

Unexpected antibodies may be clinically significant and may cause decreased red cell survival as the result of hemolytic transfusion reaction, hemolytic disease of the fetus or newborn, or autoimmune hemolytic anemia.

## 13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

## 14. LIMITATIONS OF METHOD

### 14.1 Analytical Measurement Range (AMR)

N/A

### 14.2 Precision

- This test system will not detect IgM or IgG 4 subclass antibodies. Some IgM antibodies may be detected if the Capture-R Indicator Cells carry the antigen to which the IgM antibody is directed
- Strip wells should not be used if the humidity indicator shows the presence of moisture by turning from blue to pink.
- Strip wells removed from the pouches should start incubation as soon as possible. Capture assays differ on the maximum amount of time that may elapse between pouch removal and incubation. Reference the manufacturer's insert for each strip for the exact time period.
- All reagents should be brought to room temperature (18-30°C) before testing. Erroneous test results may occur if reagents are cold.

- It is normal for indicator cells to aggregate slightly at 1-10°C storage. It is important to disperse this aggregation with multiple gentle inversions.
- Reactions between an antibody and its antigen may be weakened if acidic, unbuffered saline is used to wash red cells prior to the addition of the indicator cells. Salines of pH 6.5 or lower may cause loss of immobilized membranes. Isotonic saline must be buffered at pH 6.5-7.5 for optimal reactivity to occur between an antibody and its antigen. pH should be verified by using pH paper.
- Each centrifugation run must include at least one set of control reagents to ensure that the strip well(s) have been centrifuged and/or washed properly. This means that each batch must have acceptable positive and negative control results in order to consider the test batch valid.
- Once Capture LISS has been dispensed into the wells, the plates must be tested within one hour. Falsely positive and falsely negative results will be obtained with strip wells that have been left standing with LISS in the wells for periods beyond one hour before testing.
- The incubation time and temperature are critical to assay performance.
- Erroneous test results will occur if too few or too many Indicator Red Cells are added. Care must be taken when dispensing Indicator Red Cells. Hold the provided dropper at a 45° angle to ensure that one complete drop is added to each well. The drop size must be consistent from well to well.
- Inadequate washing of test wells will cause erroneous results.
- False positive results may be caused by:
    - Reagents not brought to room temperature prior to testing
    - Use of contaminated specimen
    - Use of contaminated reagents
    - Use of specimens drawn into neutral gel separators
    - Specimen not centrifuged properly
    - Too few indicator cells added
    - Improper instrument parameters/settings
    - Undercentrifugation of strip wells
    - Procedure not followed
- False negative results may be caused by:
    - Reagents not brought to room temperature prior to testing
    - Failure to add plasma or insufficient volume of plasma
    - Failure to add LISS or Indicator Cells
    - Failure to wash all wells properly
    - Specimen improperly stored
    - Failure to decant/aspirate saline sufficiently following last wash
    - Failure to adjust washing device properly
    - Failure to apply proper manual wash technique
    - Use of neutralized reagent indicator red cells
    - Too many indicator cells added
    - Use of contaminated reagents
    - Use of contaminated or acidic saline

- Improper instrument parameters/settings
- Overcentrifugation
- Under- or over-incubation
- Procedure not followed

### 14.3 Interfering Substances

Severe hemolysis, icterus, or lipemia can interfere with the test system.

### 14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

## 15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental, Health and Safety (EHS) Manual to the learn requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

**Report all accidents and injuries to your supervisor or the Environmental, Health and Safety Coordinator.**

## 16. RELATED DOCUMENTS

SOP: Sample Specifications for Blood Bank Testing
SOP: Quality Control Failure Resolution
SOP: Immucor CSW 100 Capture Strip Well Washer
SOP: Manual Wash Techniques
SOP: Immucor Incubator P2
SOP: Antibody Identification

## 17. REFERENCES

1. Capture Guide, Version 200, Immucor, Inc. Norcross, GA. 7/05.
2. Roback, J.D., Combs, M.R., Grossman, B.J., Hillyer, C.D. 2008. Technical Manual of the AABB, 16th ed. AABB Publishing, Bethesda, Maryland.
3. 2011. Standards for Blood Banks and Transfusion Services, 27th ed. AABB Publishing, Bethesda, Maryland.
4. Capture-R Ready-Screen (3) Manufacturer's Instructions, Insert Code 387-2, Rev 9/10. ImmucorGamma, Norcross, GA.
5. Capture-R Positive and Negative Control Sera Manufacturer's Instructions, Insert Code 352-5, Rev 12/05. ImmucorGamma, Norcross, GA.

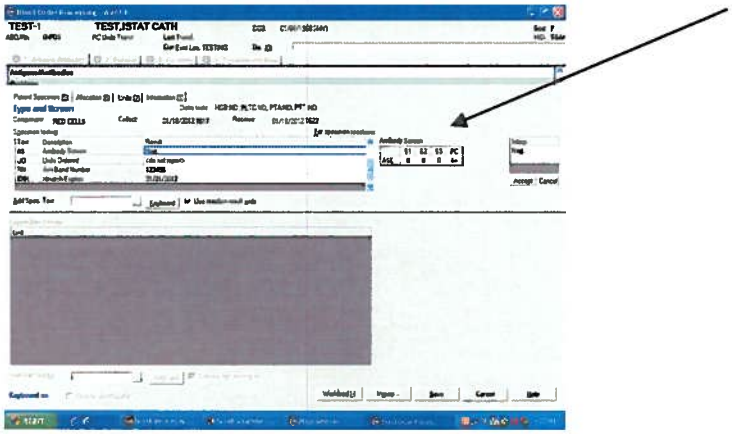
## 18. REVISION HISTORY

| Version | Date | Section | Reason | Reviser | Approval |
|---|---|---|---|---|---|
| 000 | 1.24.12 | 19 | Add A: Updated LIS data entry fields. | SCodina | NCacciabeve |
| 001 | 10.23.15 | 8 & 10 | Added a requirement to write a PI/Variance if the positive control fails (step 16 and repeat criteria). | SCodina | NCacciabeve |
| | | Footer | Version # leading zero's dropped due to new EDCS in use as of 10/7/13. | LBarrett | |
| | | | | | |

## 19. ADDENDA

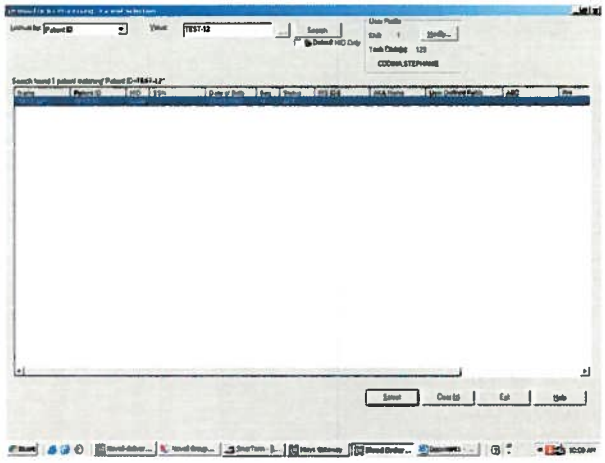
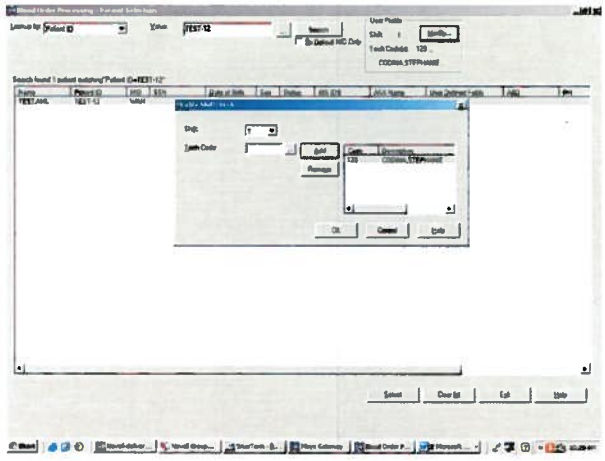
A: Resulting Antibody Screen Testing in Sunquest
B: Adding a Tech Code to the Testing Record

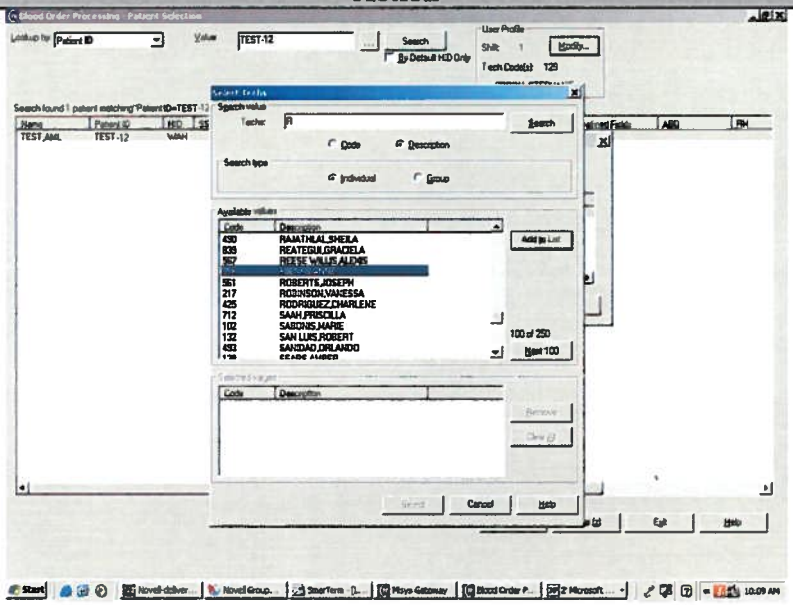
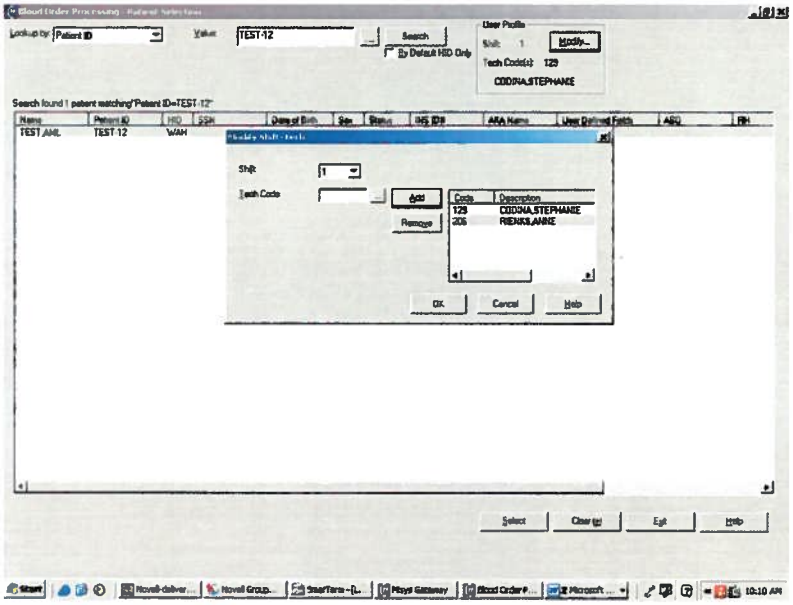
## Addendum A
## Resulting Antibody Screen Testing in Sunquest

| Step | Action |
|---|---|
| 1 | Access Sunquest function "Blood Order Processing." |
| 2 | In the "Lookup by" prompt, click on the dropdown menu and select "Patient ID." |
| 3 | In the "Value" prompt, type the patient's medical record number and click on the "Search" button. |
| 4 | If more than one patient appears, select the correct patient by clicking on the name. |
| 5 | Click the "Search all" button. |
| 6 | Click on the sample with the correct accession number. |
| 7 | Click in the "AS" (Antibody Screen) field. |
| 8 | Press the "Home" key to move your cursor to the reaction entry grid. |
| 9 | Enter the result of each screen cell in the appropriate grid box.<br>A. "S1" = Screen Cell I<br>B. "S2" = Screen Cell II<br>C. "S3" = Screen Cell III<br>D. "PC" = Positive Control<br>  <br>Keypad Map for Result Reactions<br>7 **H** \| 8 **RL** \| 9 **NT**<br>4 **4+** \| 5 **M+** \| 6 **MF**<br>1 **1+** \| 2 **2+** \| 3 **3+**<br>0 **0** \| . **NE**<br>H = Hemolysis<br>RL = Rouleaux<br>NT = Not tested<br>M+ = Microscopic<br>MF = Mixed field<br>NE = Neonatal backtype |
| 10 | Enter the interpretation in the interpretation field.<br>A. "N" = Negative<br>B. "P" = Positive |
| 11 | Click the "Save" button. |

## Addendum B
## Adding a Tech Code to the Testing Record

It is permissible for one tech to begin testing and another tech to read the test results. When this occurs, both tech codes should be documented in the LIS testing record.

| Step | Action |
|---|---|
| 1 | Access the patient test in function "Blood Order Processing." |
| 2 | Click on the "Modify" button in the "Patient Selection" screen.<br>  |
| 3 | If you do not know the code of the other tech, click on the ellipse button (…) to pull up a search screen.<br>A. Type in the first letter of the last name of the other tech.<br>B. Click on the "Search" button.<br>C. Select the correct tech name from the pop-up list.<br>D. Click on the "Add to List" button.<br>  |

| Step | Action |
|---|---|
| 3 Cont |  |
| 4 | Click the "OK" button.  |