## TRAINING UPDATE

| | | | |
|---|---|---|---|
| **Lab Location:** | SGAH and WAH | **Date Implemented:** | 1.21.2013 |
| **Department:** | Blood Bank | **Due Date:** | 1.31.2013 |

## DESCRIPTION OF PROCEDURE REVISION

| **Name of procedure:** |
|---|
| ABO/Rh Typing (Manual Tube) |
| **Description of change(s):** |

- Weak D instructions were removed from the ABO/Rh procedure and moved to a new, Weak D procedure.
- We will now report and interpret the immediate spin D result separately from the weak D result.
  - Report as Rh-positive if IS anti-D result 2+, 3+, or 4+
  - Report as Rh-negative if IS anti-D result is 0
  - Report as Rh-inconclusive if IS anti-D result is W+ or 1+
  - Do NOT wait for weak D results prior to reporting Rh results
  - DO NOT change the ABO/Rh type from negative to positive if the weak D is positive

The ACOG (American Congress for Obstetrics and Gynecology) is recommending that labs interpret and report immediate spin and weak D results separately. There is no standard process between labs for performing and reporting weak D testing. As a result, different labs report Rh results differently for the same patients. The ACOG advises that labs let the physicians interpret these results to ensure the patient gets RhIG whenever indicated. Our OB/Gyn physicians voted to use the ACOG recommendations.

Technical SOP

| | | |
|---|---|---|
| **Title** | **ABO/Rh Typing (Manual Tube)** | |
| **Prepared by** | Stephanie Codina | Date: 1.13.2012 |
| **Owner** | Stephanie Codina | Date: 1.13.2013 |

| **Laboratory Approval** | **Local Effective Date:** | |
|---|---|---|
| **Print Name and Title** | **Signature** | **Date** |
| *Refer to the electronic signature page for approval and approval dates.* | | |
| | | |
| | | |
| | | |
| | | |

| **Review** | | |
|---|---|---|
| **Print Name** | **Signature** | **Date** |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

Form revised 10/31/02

## TABLE OF CONTENTS



## 1. TEST INFORMATION

| Assay | Method/Instrument | Order Code | Local Code |
|---|---|---|---|
| ABO blood group | Tube test | N/A | N/A |
| Rh type | Tube test | N/A | N/A |

| Synonyms/Abbreviations |
|---|
| Group and Type, Blood type |

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

Form revised 10/31/02

## 2. ANALYTICAL PRINCIPLE

A patient's red cell suspension mixed with certain antisera will demonstrate the presence or absence of agglutination. Likewise, the mixing of a patient's serum with certain red cell suspensions will demonstrate the presence or absence of agglutination. The subsequent pattern of agglutination and lack of agglutination is utilized to determine the ABO group.

The descriptive terms Rh-positive and Rh-negative refer to the presence or absence of the red cell antigen D. Red cell suspensions that show agglutination when mixed with anti-D reagent are classified as Rh-positive. Red cells that show no agglutination, either immediately or with anti-human globulin, are termed Rh-negative.

## 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. Refer to procedure "Sample Specifications for Blood Bank Testing" for details. |

### 3.2 Specimen Type & Handling

| Criteria | | |
|---|---|---|
| **Type -Preferred**<br>**-Other Acceptable** | Red cells and plasma (EDTA)<br>Heparin tube or clotted sample in tube w/out serum separator gel | |
| **Collection Container** | Lavender top tube, dark green top tube, or red top tube (without serum separator). | |
| **Volume - Optimum**<br>**- Minimum** | 10ml<br>2ml | |
| **Transport Container and Temperature** | Same as above, at room temperature | |
| **Stability & Storage Requirements** | Room Temperature: | 24 hours |
| | Refrigerated: | EDTA samples <10 days,<br>Clotted samples <21 days |
| | Frozen: | Unacceptable |
| **Timing Considerations** | Test as soon as possible following collection | |

Form revised 10/31/02

| Criteria | |
|---|---|
| **Unacceptable Specimens & Actions to Take** | Frozen, Incomplete or incorrect labeling – refer to procedure "Sample Specifications for Blood Bank Testing" for details. |
| **Compromising Physical Characteristics** | Refer to section 14. |
| **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.

**The package insert for a new lot of reagents must be reviewed for any changes before the reagent is used. A current package insert is available in the Reagent Insert binder.**

### 4.1 Reagent Summary

| Reagents / Kits | Supplier & Catalog Number |
|---|---|
| Anti-A | Immucor Cat. #6400 or equivalent |
| Anti-B | Immucor Cat. #6406 or equivalent |
| Anti-D, Series 4 | Immucor Cat. #6412 or equivalent |
| 22% Albumin | Immucor Cat. #2327 or equivalent |
| 2-4% Reagent Red Blood Cells (Group $A_1$ and B cells) | Immucor Cat. #2345 or equivalent |

### 4.2 Reagent Preparation and Storage

| | |
|---|---|
| **Reagent** | Anti-A, Anti-B, Anti-D, Albumin |
| **Preparation** | Ready to use as supplied. |
| **Storage** | 1-10°C |
| **Stability** | Stable until manufacturer's expiration date. |
| **Special Handling** | Do not use if turbid - indicates deterioration or contamination. |

| | |
|---|---|
| **Reagent** | Reagent Red Blood Cells (Group $A_1$ and B cells) |
| **Preparation** | Resuspend red cells before use by gently inverting each vial several times. |
| **Storage** | 1-10°C |
| **Stability** | Stable until manufacturer's expiration date. |
| **Special Handling** | None |

Form revised 10/31/02

## 5. CALIBRATORS/STANDARDS

N/A

## 6. QUALITY CONTROL

### 6.1 Controls Used

| Controls | Supplier and Catalog Number |
|---|---|
| Ortho Confidence Control Kit | Ortho Clinical Diagnostics, Cat. #32418 |

### 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.**

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. Refer to the control insert sheet for preparation, storage and handling instructions.

### 6.3 Frequency

Daily

### 6.4 Tolerance Limits

Refer to procedure "Daily Reagent Quality Control."

### 6.5 Review Patient Data

N/A

### 6.6 Documentation

Refer to procedure "Daily Reagent Quality Control."

### 6.7 Quality Assurance Program

- Participation in CAP proficiency testing.
- Each new shipment and lot number of reagent is tested with control materials before being placed into use. Reagents that do not perform as expected are not placed into use.

## 7. EQUIPMENT and SUPPLIES

### 7.1 Assay Platform

N/A

### 7.2 Equipment

Serological centrifuge
Agglutination Viewer

### 7.3 Supplies

12 x 75 mm test tubes and rack
Transfer pipettes
Saline, 0.9%

## 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.**

| Step | Action |
|---|---|
| 1 | Confirm specimen acceptability and specimen labeling per procedure, "Sample Specifications for Blood Bank Testing." |
| 2 | Label six tubes with the patient or unit identifiers (label one extra tube if the patient is known AB-positive). Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing."<br>A. Patient identifiers include the patient's first and last initials or the first 3 letters of the patient's last name.<br>B. Unit identifiers include the last 3 digits of the unit number.<br>C. Additional identifiers must be used if needed to differentiate between patients or units. |

| Step | Action |
|---|---|
| 3 | Label one tube with each tube with one of the following:<br>A. "A"<br>B. "B"<br>C. "D"<br>D. "A1C"<br>E. "BC"<br>F. "CON" (only for AB-positive patients)<br>The remaining tube will be used for the red cell suspension of the test sample. Labeling will only contain patient identifiers, because no test reagent will be added. |
| 4 | Add one drop of the blood grouping reagent to the appropriately labeled tube.<br>A. Add 1 drop of Anti-A to the tube labeled "A."<br>B. Add 1 drop of Anti-B to the tube labeled "B."<br>C. Add 1 drop of Anti-D to the tube labeled "D."<br>D. Add 1 drop of 22% albumin to the tube labeled "CON." |
| 5 | Add two drops of patient plasma to each tube labeled "A1C" or "BC." |
| 6 | Gently invert reverse grouping cell vials to resuspend completely. |
| 7 | Add one drop of the reverse grouping cells to the appropriately labeled tube.<br>A. Add one drop of the $A_1$ cell to the tube labeled "A1C."<br>B. Add one drop of the B cell to the tube labeled "BC." |
| 8 | Prepare a 2-4% suspension of patient or donor red cells in isotonic saline in the remaining tube per procedure, "Preparing a 2-4% Cell Suspension for Testing." |
| 9 | Add one drop of the patient cell suspension to the tubes labeled "A," "B," "D," and "CON" (if applicable). |
| 10 | Mix each tube thoroughly and perform a visual check to ensure reagent volume is correct in each tube. |
| **NOTE: The remaining steps should be performed for ONE patient at a time. Complete steps 11-14 for one patient and then return to step 11 for the next patient.** | |
| 11 | Serofuge for the saline phase calibration time listed on the serofuge. |

Form revised 10/31/02

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>12</td><td>Access the patient information data entry screen in Sunquest using function "Blood Order Processing" or utilize a computer downtime form.</td></tr>
<tr><td>13</td><td>Remove the tubes from the serofuge and verify the labeling of the tubes pulled from the serofuge matches the patient information in the computer or on the downtime form.</td></tr>
<tr><td>14</td><td>Gently resuspend each cell button one tube at a time. Read each tube macroscopically for hemolysis and/or agglutination using an agglutination viewer. Immediately record results in the computer or on a blood bank worksheet. Refer to procedure, "Blood Bank Reaction Grading."</td></tr>
<tr><td>15</td><td>If the patient appears to be AB-positive and the D control was not run, return to step 2 and perform a D control to rule out polyagglutination.</td></tr>
<tr><td>16</td><td>Compare the current ABO results with any historical ABO results.
<table>
<tr><th>If a historical record…</th><th>And the current and historical results…</th><th>Then…</th></tr>
<tr><td rowspan="3">Exists</td><td>Agree</td><td>Interpret results in the LIS or on a downtime form.</td></tr>
<tr><td>Do not agree due to ABO</td><td>Refer to procedure, "ABO Discrepancies with Historical Data."</td></tr>
<tr><td>Do not agree due to Rh</td><td>Refer to procedure, "Weak D Typing."</td></tr>
<tr><td>Does not exist</td><td>N/A</td><td>Request a second sample for ABO confirmation per procedure, "Confirmation of Patient's Blood Type (ABO Recheck)."</td></tr>
</table></td></tr>
</table>

## 9. CALCULATIONS

N/A

## 10. REPORTING RESULTS AND REPEAT CRITERIA

### 10.1 Interpretation of Data

Agglutination represents a positive result in a particular tube while no agglutination represents a negative reaction in a particular tube.

### Minimum Reaction Strength

ABO forward and reverse typing reactions must have strengths of ≥2+ to be valid. Reaction strengths <2+ may indicate the reaction is due to an antibody other than Anti-A or Anti-B and cannot be interpreted without additional workup. Refer to procedure, "ABO Discrepancies."

## 10.2 Interpretation of Data

### ABO/Rh Results

| Reaction of Cells Tested With | | | | Reaction of Serum or Plasma Tested With | | Interpretation |
|---|---|---|---|---|---|---|
| Anti-A | Anti-B | Anti-D | 22% Bovine Albumin | A1 Cell | B Cell | |
| 0 | 0 | 0 | N/A | ≥2+ | ≥2+ | O-negative |
| 0 | 0 | ≥2+ | N/A | ≥2+ | ≥2+ | O-positive |
| ≥2+ | 0 | 0 | N/A | 0 | ≥2+ | A-negative |
| ≥2+ | 0 | ≥2+ | N/A | 0 | ≥2+ | A-positive |
| 0 | ≥2+ | 0 | N/A | ≥2+ | 0 | B-negative |
| 0 | ≥2+ | ≥2+ | N/A | ≥2+ | 0 | B-positive |
| ≥2+ | ≥2+ | 0 | N/A | 0 | 0 | AB-negative |
| ≥2+ | ≥2+ | ≥2+ | 0 | 0 | 0 | AB-positive |
| ≥2+ | ≥2+ | ≥2+ | + (any strength) | 0 | 0 | Invalid |
| ≥2+ | ≥2+ | ≥2+ | Not Tested | 0 | 0 | Invalid |
| Any | Any | Weak Pos or 1+ | Any | Any | Any | Rh-Indeterminate |
| Any reaction that is positive and <2+ in strength requires additional testing. For ABO, refer to procedure "ABO Discrepancies." For Rh, refer to "Weak D" section of this procedure. | | | | | | |

Form revised 10/31/02

### 10.3 Discrepancies between ABO forward and reverse group

| Step | Action |
|---|---|
| 1 | Refer to procedure, "ABO Discrepancies" if the blood type interpretation cannot be made based on the above table. |
| 2 | **Do not issue type specific blood products to any patient until an ABO discrepancy is resolved.** |
| 3 | **Issue only group O red blood cells and group AB plasma products if a patient Requires transfusion before testing can be completed.** |

### 10.4 Donor (Unit) ABO/Rh

Refer to procedure "Entering Blood Products Into Inventory."

## 11. EXPECTED VALUES

N/A

## 12. CLINICAL SIGNIFICANCE

N/A

## 13. PROCEDURE NOTES

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

## 14. LIMITATIONS OF METHOD

| | |
|---|---|
| 1 | Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test reagents. |
| 2 | Certain subgroups of A and B may produce reactions that are weaker than those routinely obtained with A or B cells. Depending on the subgroup involved, some may appear non-reactive. |
| 3 | In certain clinical situations, Group A red cells may acquire a B-like antigen in vivo due to the action of bacterial enzymes. Some Anti-B reagents of monoclonal or polyclonal origin can react with these cells. In cases where the results of this Anti-B reagent are questionable, further testing is required. Refer to the procedure "ABO Discrepancies." |
| 4 | Reverse grouping cells possess blood group antigens other than A or B. It is possible for a serum sample to contain a saline phase agglutinin that interferes with reverse grouping tests. |

| | |
|---|---|
| 5 | Forward and reverse grouping tests are to be performed and compared before interpretations are made. Stronger reactions cannot be assumed to be more correct than weaker ones. |
| 6 | Acid-dependent autoagglutinins are a potential source of false-positive agglutination in tests with this Anti-B reagent and unwashed cells. The same nonspecific reactivity may not be seen with Anti-A. Repeat testing with washed cells may resolve the problem. |
| 7 | Cord blood samples may contain maternal anti-A and/or anti-B and are not used routinely for reverse grouping. |
| 8 | Do not use murine monoclonal reagents in indirect antiglobulin tests using antihuman globulin reagents. |
| 9 | Positive reactions obtained with stored specimens may be weaker than those obtained with fresh specimens. |
| 10 | If a patient has received blood of an Rh-type other than his/her own, it may be difficult to determine the correct Rh type. If a patient with a history of typing as Rh-negative is found to be typing as Rh-positive, obtain a history to see if the patient has received Rh-positive blood. D-negative blood should be transfused if there is any question about the patient's Rh-type. |
| 11 | In certain situations, falsely positive results may be obtained in direct tests with anti-D. The D+ red cells of most people will produce strong reactions (3-4+) with monoclonal anti-D. Reactions of less than 2+ in immediate spin tests should be thoroughly evaluated since such reaction may not be due to interaction between reagent anti-D and the D antigen on the test red cells. |
| 12 | The presence of strong cold agglutinins or strong rouleaux-forming factors in the serum of a patient could lead to cellular aggregation in tests employing unwashed plasma- or serum-suspended red cells that may be interpreted as agglutination. The same factors often lead to discrepant results in ABO grouping tests using similarly prepared red cells. To determine the validity of positive tests obtained in the presence of potent cold agglutinins or rouleaux-forming proteins, a control of 6–30% albumin should be tested in parallel. Positive results obtained in control tests indicate that those obtained with Anti-D may be invalid. To overcome such problems, test red cells should be thoroughly washed in warm saline and resuspended in saline before testing. |

## 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 learn the 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: Daily Reagent Quality Control
SOP: Sample Specifications for Blood Bank Testing
SOP: Preparing a 2-4% Cell Suspension for Testing
SOP: Blood Bank Reaction Grading
SOP: ABO Discrepancies
SOP: ABO Discrepancies with Historical Data
SOP: Weak D Typing
SOP: Confirmation of Patient's Blood Type (ABO Recheck)

## 17. REFERENCES

1. Roback, J.D., Grossman, B.J., Harris, T., and Hillyer, C.D. 2011. Technical Manual of the AABB, 17th ed. AABB Publishing, Bethesda, Maryland.
2. Standards for Blood Banks and Transfusion Services, 2012. AABB, 28th ed. AABB Publishing, Bethesda, Maryland.
3. Package Insert for Anti-A, Anti-B (Murine Monoclonal), Anti-A,B (Murine Monoclonal blend), Immucor Inc., Norcross, GA, Insert Code 3006-1, Revision Date 10/2007.
5. Package Insert for Reagent Red Blood Cells Referencells, Immucor Inc., Norcross, GA, Insert Code 300-15, Revision Date 10/2007.
6. Package Insert for Anti-D, Series 4 (Monoclonal Blend), ImmucorGamma, Norcross, GA, Insert 336-8, 8/07.

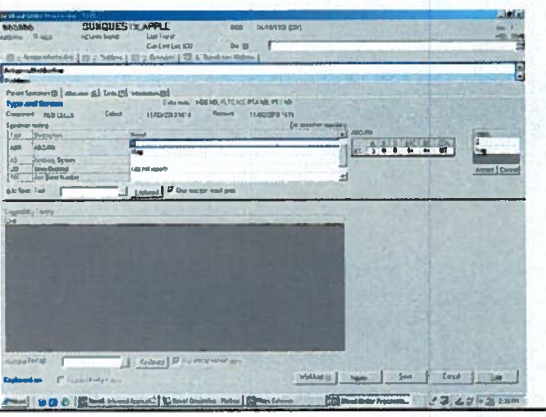
## 18. REVISION HISTORY

| Version | Date | Section | Reason | Reviser | Approval |
|---|---|---|---|---|---|
| | | | Supersedes SGAH.BB53.000, WAH.BB51.000 | | |
| | | | | | |
| | | | | | |
| | | | | | |

## 19. ADDENDA

Appendix A: LIS Entry of ABO/Rh

## Appendix A
## LIS Entry of ABO/Rh

| 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 on the "Search All" button. |
| 6 | Click on the sample with the correct accession number. |
| 7 | Click in the ABR (ABO/Rh) result entry field. |
| 8 | Press the "Home" key to move your cursor to the reaction entry grid. |
| 9 | Enter the result of each tube in the appropriate grid box. See the Keypad Map below for specific key entry.<br>A. A = Anti-A<br>B. B = Anti-B<br>C. D = Anti-D<br>D. A1C = $A_1$ Cell<br>E. BC = B Cell<br>F. CON = Albumin Control<br>  |

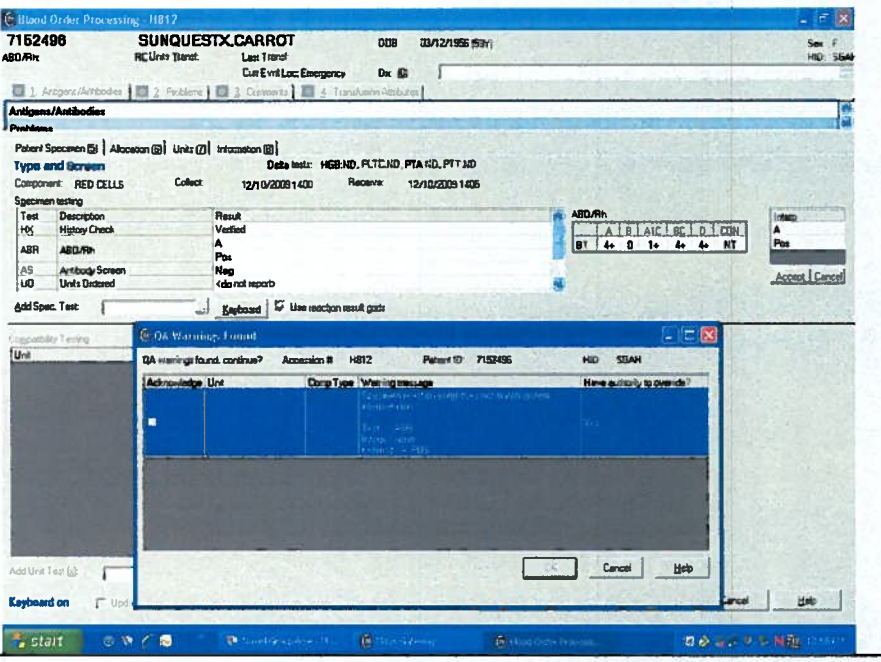
Form revised 10/31/02

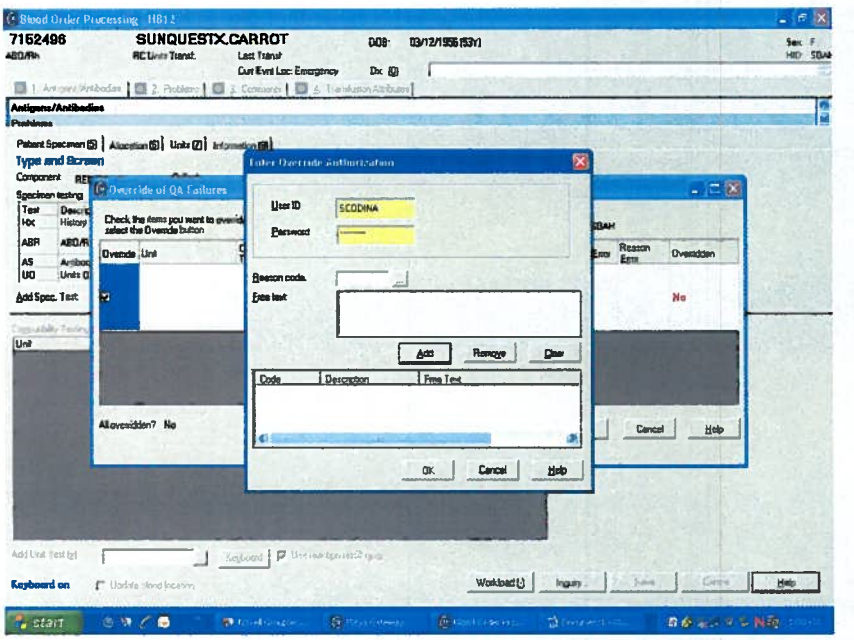
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>9<br>Cont<br>.</td><td>Keypad Map for Result Reactions<br><table><tr><td>7<br>H</td><td>8<br>RL</td><td>9<br>NT</td></tr><tr><td>4<br>4+</td><td>5<br>M+</td><td>6<br>MF</td></tr><tr><td>1<br>1+</td><td>2<br>2+</td><td>3<br>3+</td></tr><tr><td colspan="2">0<br>0</td><td>.<br>NE</td></tr></table>H = Hemolysis<br>RL = Rouleaux<br>NT = Not tested<br>M+ = Microscopic<br>MF = Mixed field<br>NE = Neonatal backtype</td></tr>
<tr><td>10</td><td>Enter the blood type in the interpretation field. Note: ABO results are generally not interpreted until resolution if an ABO discrepancy exists.<br><br>Keyboard for ABO/Rh Interpretation<br><table><tr><th>Keyboard</th><th>Sunquest Translation</th></tr><tr><td>A</td><td>Group A</td></tr><tr><td>B</td><td>Group B</td></tr><tr><td>C</td><td>Group AB</td></tr><tr><td>O</td><td>Group O</td></tr><tr><td>N</td><td>Negative</td></tr><tr><td>P</td><td>Positive</td></tr><tr><td>I</td><td>Indeterminate</td></tr></table></td></tr>
<tr><td>11</td><td>Click on the “Save” button.</td></tr>
</table>

**Quality Assurance (QA) Failure Messages**

| Step | Action |
|---|---|
| 1 | Sunquest compares patterns of the reactions entered to those that are defined in the system. A QA Warning message will appear on the screen if the entered results do not match a defined pattern and interpretation. |

| Step | Action |
|---|---|
| 2 | If a QA Warning message appears on the screen, verify that the reaction results ane interpretation are entered correctly. |



| Step | Action |
|---|---|
| 3 | A. If a clerical error exists, correct results and click "SAVE" again.<br>B. If the results were entered correctly, make sure you understand the QA warning message. If you understand why the computer is flagging the results or interpretation (ABO discrepancy), you can override the QA failure. **Do not interpret any ABO group that has a discrepancy until the discrepancy is resolved.**<br>a. Click on the "Acknowledge" box and click "OK."<br>b. When you save your results, the QA Warning will appear again.<br>i. Click on the "Override" box and click "OK."<br>ii. Type in your user ID and password.<br>iii. Type in an ETC (English text code) to indicate why you are accepting the QA failure or type a reason in the "Freetext" box.<br>iv. Click the "Add" button.<br>v. Click on "OK."<br>  |
| 4 | All overridden QA failures will print on the Quality Assurance report that is reviewed daily. |

Form revised 10/31/02