STEM CELL LABORATORY (STCL)

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ABO Rh Typing

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STCL-SOP-049
ABO/RH TYPING

1 PURPOSE

1.1 To provide instructions needed to perform an ABO/Rh type in a test tube for a cellular product.

2 INTRODUCTION

2.1 Forward typing requires the use of known anti-A, anti-B, and anti-D sera to demonstrate the presence or absence of antigens on unknown cells. Reserve typing (when indicated) requires the use of known cell suspensions to demonstrate the presence or absence of antibodies in serum or plasma.

3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Director, Laboratory Management, Quality System Unit, and designated laboratory testing personnel are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1 STCL Stem Cell Laboratory

5 MATERIALS

5.1 Specimens

- 5.1.1 Bone Marrow
- 5.1.2 PBPC by apheresis
- 5.1.3 Granulocytes
- 5.1.4 Cord blood

5.2 Reagents

- 5.2.1 Reverse typing cells: A_1, B, (A_2 if indicated)
- 5.2.2 Routine antisera: Anti-A, Anti-B, Anti-D

5.3 Supplies

- 5.3.1 12 x 75 mm test tube
- 5.3.2 Disposable pipettes
- 5.3.3 Cell pack

6 EQUIPMENT

6.1 Centrifuge

6.2 Agglutination mirror

7 SAFETY

7.1 Wear all appropriate personal protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to gloves, lab coats, etc.

8 PROCEDURE

8.1 Prior to performing samples each day, reagent quality control should be performed and recorded on the Blood Bank worksheet (see *STCL-SOP-049 (FRM1)*. Forward grouping is initially performed using an anticoagulated sample. Subsequent typing may be performed on cells obtained from a clotted sample.

NOTE: *QC results must be reviewed for acceptability before reporting cellular product confirmatory test results. The Blood Bank Worksheet (STCL-SOP-049 FRM1) should reflect the date and initials of the person who reviewed the QC results for accuracy. QC reviews should be completed daily whenever testing is performed.*

8.2 Forward typing and D typing

8.2.1 Label a test tube with first three (3) letters of last name or attach the ISBT barcode of the product being tested. Prepare a 4% saline suspension of recipient/donor cells by mixing ½ ml of cell pack and 3-4 drops of recipient/donor sample.

8.2.2 Label three (3) test tubes with first three (3) letters of last name or ISBT 128 bar code and antibody used in each tube (i.e. A, B, or D).

Example:	Last name is Jones			or	W158209210000A
	Jon	Jon	Jon		W158209210000B
	A	B	D		W158209210000D

8.2.3 Place one (1) drop of appropriate anti-sera into each corresponding tube.

8.2.4 Add one (1) drop of recipient/donor 4% red cell suspension to each tube.

8.2.5 Mix well.

8.2.6 Centrifuge tubes for the designated amount of time for saline reactions as indicated on the label affixed to each centrifuge.

8.2.7 Using the agglutination mirror, score and record agglutination reactions/ hemolysis (using the tables below) on the Blood Bank worksheet as well as on the appropriate processing worksheet. (**NOTE**: IF hemolysis is observed, contact the Medical Director or designee for further instructions.)

8.2.8 Perform additional testing, *if indicated.*

8.2.8.1 All AB positive recipients must have an AB control tested (see *STCL-SOP-049 (JA1)* AB Serum Test (AB Control). If the AB control test is positive, consult Transfusion Services for additional testing recommendations.

8.3 Reverse, or serum, grouping (*ONLY if ordered or indicated*)

8.3.1 Label two (2) test tubes with first three (3) letters of last name or ISBT 128 bar code and A1 cells or B cells.

Example: Last name is Jones or W158209210000a
Jon Jon Jon W158209210000b
a b

8.3.2 Add two (2) drops of recipient/donor serum or plasma to each tube.

8.3.3 Add one (1) drop of A1cells to tube labeled "a" and one (1) drop of B cells to tube labeled "b".

8.3.4 Mix well.

8.3.5 Centrifuge tubes for the designated amount of time for saline reactions as indicated on the label affixed to each centrifuge.

8.3.6 Using the agglutination mirror, score and record agglutination reactions / hemolysis (using the tables below) on the Blood Bank worksheet as well as on the appropriate processing worksheet. (**NOTE**: IF hemolysis is observed, contact the Medical Director or designee for further instructions.)

8.4 Discrepant results

8.4.1 The majority of the cellular products tested in the STCL have already been ABO Rh typed elsewhere and those historical results have been provided to the STCL. If confirmatory typing results obtained in the STCL from the cellular product do **NOT** match the historical ABO Rh types provided, a sample can be sent to the Transfusion Services for investigation using *STCL-SOP-049 FRM2*.

8.4.2 Historical ABO Rh results are **NOT** available from newborn cord blood units received in the STCL for processing. Any Rh Negative sample is sent to the Transfusion Services for confirmatory testing (Weak D testing, etc). When sending specimens to the Transfusion Services for confirmatory testing, be sure to send *STCL-SOP-049 FRM2* along with the specimen(s).

8.4.3 Any ABO Rh discrepancy that can NOT be resolved in a timely manner should be brought to the attention of the medical director (or designee), lab manager, etc.

NOTE:
Only the results of the testing performed will be recorded on the Blood Bank worksheet (*STCL-SOP-049 FRM1*). All other result columns will be left BLANK.

AGGLUTINATION TABLE

Strength of Reaction	Cell Button Description	Background Description
4+	Remains in one clump	Clear
3+	Several small clumps	Clear
2+	Many small clumps, equal size	Hazy to clear
1+	Finely granular definite small clumps	Turbid

MF = mixed field

HEMOLYSIS TABLE

Description of Hemolysis	Definition of Cell Button	Background Color
Gross (GH)	Marked reduction in cell button size	Dark red
Moderate (MH)	Slight Reduction in cell button size	Obvious Red
Slight (SH)	No reduction in cell button size	Pink tinge

GH = Gross Hemolysis
MH = Moderate Hemolysis
SH = Slight Hemolysis

TABLE 1
ABO – Forward Grouping

Interpretation – Reaction with known antisera

Anti-A	Anti-B	Blood Group
0	0	0
1	0	A
2	0	A
3	0	A
4	0	A
0	1	B
0	2	B
0	3	B
0	4	B
1	1	AB
2	1	AB
3	1	AB
4	1	AB
1	2	AB
2	2	AB
3	2	AB
4	2	AB
1	3	AB
2	3	AB
3	3	AB
4	3	AB
1	4	AB
2	4	AB
3	4	AB
4	4	AB

TABLE II
ABO – Reverse Grouping

(Performed ONLY if ordered or indicated)

Interpretation – Reaction with known cells

A_1 cells	B cells	Blood Group
1 - 4	1 - 4	O
0	1 - 4	A
1 - 4	0	B
0	0	AB

9 RELATED DOCUMENTS/FORMS

9.1 STCL-SOP-049 (FRM1) Blood Bank Worksheet

9.2 STCL-SOP-049 (FRM2) ABO Rh Confirmatory Typing Test Request

9.3 STCL-SOP-049 (JA1) AB Serum Test (AB Control)

9.4 STCL-SOP-049 (JA2) Performing Daily Control of ABO/Rh Reagents

9.5 STCL-SOP-049 (JA3) Calibration of Fixed Speed Centrifuges

10 REFERENCES

10.1 AABB Technical Manual, 16th Edition, 2008

10.2 Standards for Blood Banks and Transfusion Services, 22nd Edition

10.3 Immucor Gamma Blood Grouping Reagent Anti A and Anti B (Murine Monoclonal) Series 1, Rev 01/06

10.4 Immucor Gamma Blood Grouping Reagent Anti D (Series 4) Monoclonal Blend, Rev 08/07

10.5 Transfusion Medicine procedure "Grading Agglutination Reactions"

10.6 Immucor Gamma Reagent Red Blood Cells, Rev 10/07

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
09	B. Waters-Pick	Section 8.1 added "**NOTE**: *QC results must be reviewed for acceptability before reporting cellular product confirmatory test results. The Blood Bank Worksheet (STCL-SOP-049 FRM1) should reflect the date and initials of the person who reviewed the QC results for accuracy. QC reviews should be completed daily whenever testing is performed.*"

Signature Manifest

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STCL-SOP-049 ABO Rh Typing

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