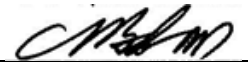
Original Effective Date:	10/2016
Last Approved Review Date:	10/2016
Last Approved Revision Date:	10/2016
Responsible Department:	Laboratory
Responsible Person:	Laboratory Medical Director

Signature: 

Fetal Hemoglobin by Flow Cytometry

Purpose

This procedure provides instructions for how to send out testing for fetal hemoglobin by flow cytometry

Background

The passage of erythrocytes from a Rh positive fetus into the circulation of a Rh negative mother results in the formation of specific Rh antibodies. In subsequent pregnancies, the Rh antibodies formed in the blood serum of the Rh negative mother are readily transmissible though the placenta into the circulation of the fetus. The action of the antibodies on the Rh postitive cells of the fetus may result in a disease entity recognized as isohemolytic desease, or erythroblastosis.

Flow Cytometry uses light that passes through or scatters and hits different points in the chamber. Based on the scattered light pattern, the cytometer program can differentiate fetal hemoglobin which has been tagged from adult hemoglobin. This test is much more precise and specific for fetal hemoglobin.

Specimen

Mother's blood

- Anticoagulated (EDTA)
- Refrigerated or ambient
- Specimens should be tested < 72 hours from collection.
- Hemolyzed specimens are not acceptable

Materials

Reagents	Supplies	Equipment
• NA	• Barnes-Jewish Flow Cytometry Immuno Phenotyping requisition • "Stat Testing" form • Biohazard bag	• NA

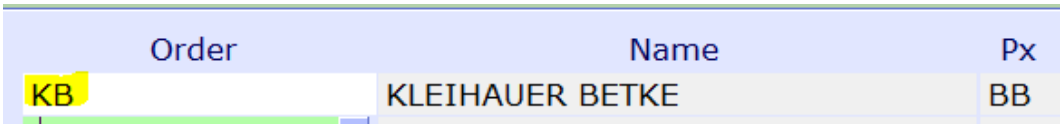
Quality Control

NA

Procedure

1. **Test Ordering**
2. **Determination of Test Flow**
3. **Computer Entry and Form Preparation**
4. **Packaging Sample**
5. **Arranging for Courier Service**
6. **Receiving and Reporting of Results**

1. Test Ordering

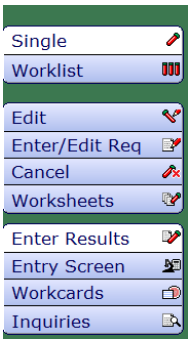
Step	Action
1	Nursing should order **Kleihauer-Betke** testing on the OM side.
2	If ordered on the lab side of MCARE, order *KB* in the **Enter/Edit** tab of the **Specimen** tab. 

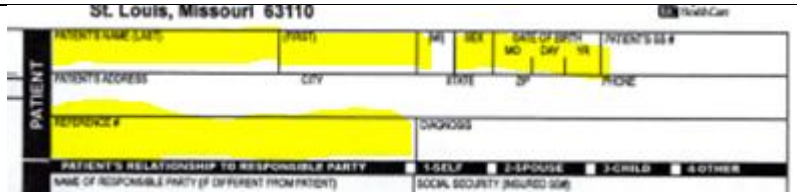
2. Determination of Test Flow

Step	Action
1	Tech must determine if the fetal hemoglobin stain will be performed or if the testing will be sent to Barnes-Jewish hospital for flow cytometry testing. • Note: all efforts should be made to send testing for flow as this is the better testing and technique for fetal hemoglobin determination.
2	Refer to the chart below.
3	**Send for Flow Cytometry if any of the following…** • M-F between 0600-1900 • Routine • For determination of RhIg to be given after normal delivery • Requested by physician **Perform FHS if any of the following….** • Weekends or holidays • After 1900 and before 0600 M-F • Doctor determines it cannot wait • It is a weekend or holiday and patient will be discharged before next regular run of flow.
4	If it is determined that the testing will be performed by staining, then refer to the *Fetal Hemoglobin Stain* procedure.
5	If the testing will be sent to Barnes-Jewish hospital, continue to next section.

3. Computer Entry and Form Preparation

Step	Action
1	Log in to MCARE

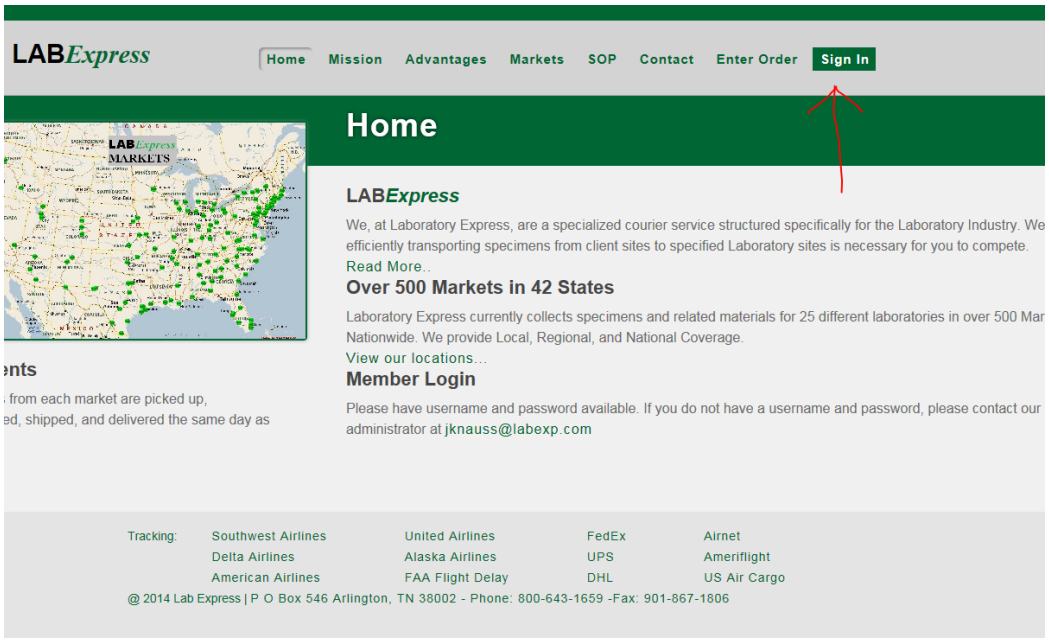
2	Go to the **Specimen** tab in the blood bank module.
3	Click on **Enter Results** 
4	Type in Specimen M#, patient name or BB# to retrieve testing.
5	Select the specimen with the correct test to result
6	Enter **FLOW KB** in the **Which Test?** field, or press F9 for options. 
7	The next field will appear which is **Previous BT?** Enter **Y** if the patient has a historical blood type or **N** if the patient does not have a historical blood type.
8	**If you entered "N"…** • The system will order an ABORH • Perform the ABORH and enter the results before proceeding. • See appropriate SOP for directions on performing and resulting ABORH. • Proceed to step 9. **If you entered "Y"….** • Proceed to step 9.
9	In the **PAT'S RH TYPE?** field, enter the patient's Rh type from the current ABORH or historical ABORH. • **N** for negative • **P** for positive
10	Press **F12** or click **Save**.
11	Obtain the *Barnes-Jewish Flow Cytometry Immuno Phenotyping* requisition form and the *BJH Stat Testing* form from the forms drawer. See *Appendix A-BJH Flow Cytometry Immuno Phenotyping Form* and *Appendix B-BJH Stat Testing Form.*
12	Fill out the following at the top of the flow req. • Patient's first and last name • Patient's sex • Patient's DOB • Patient's MRN in the space marked "Reference #"

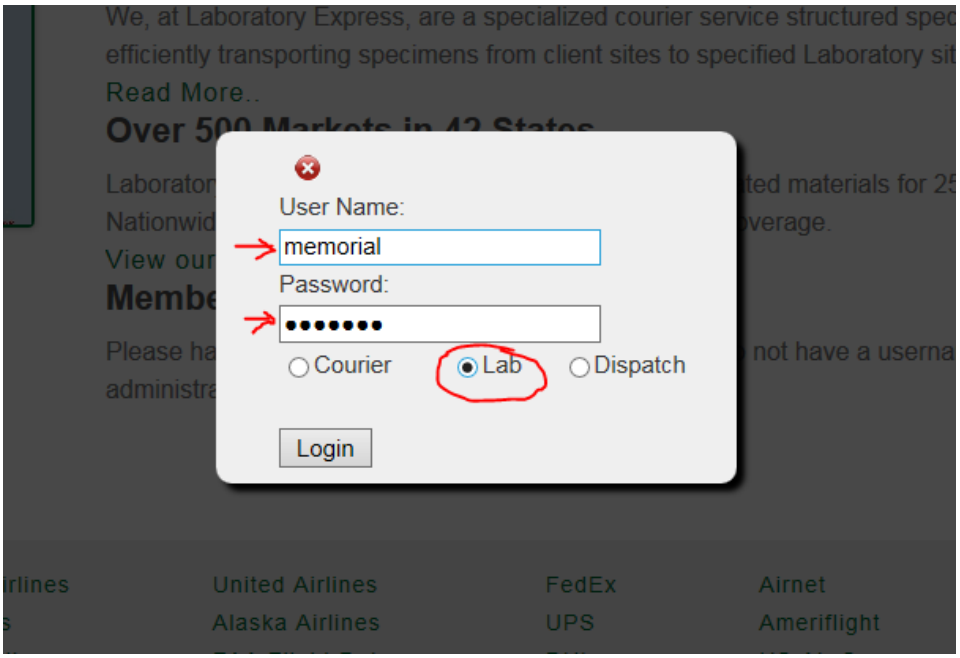
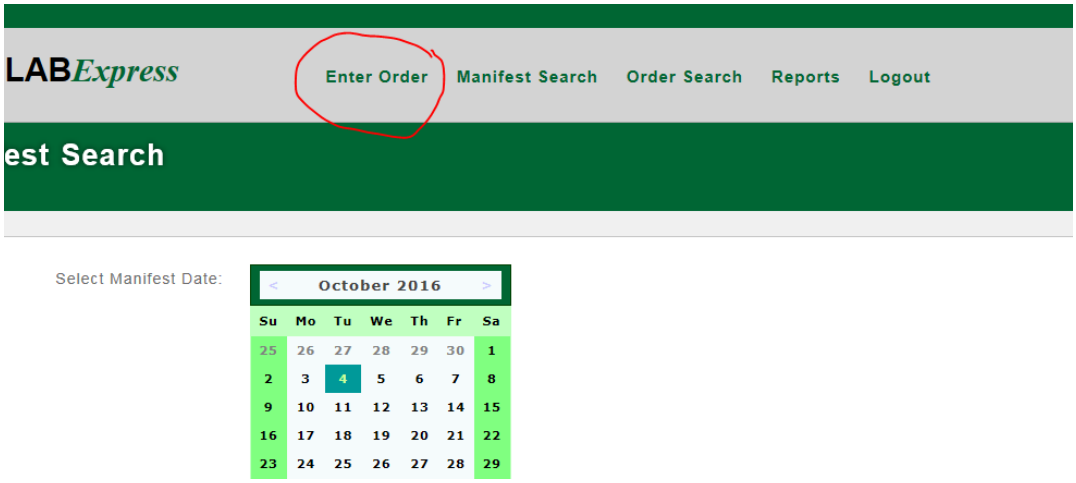
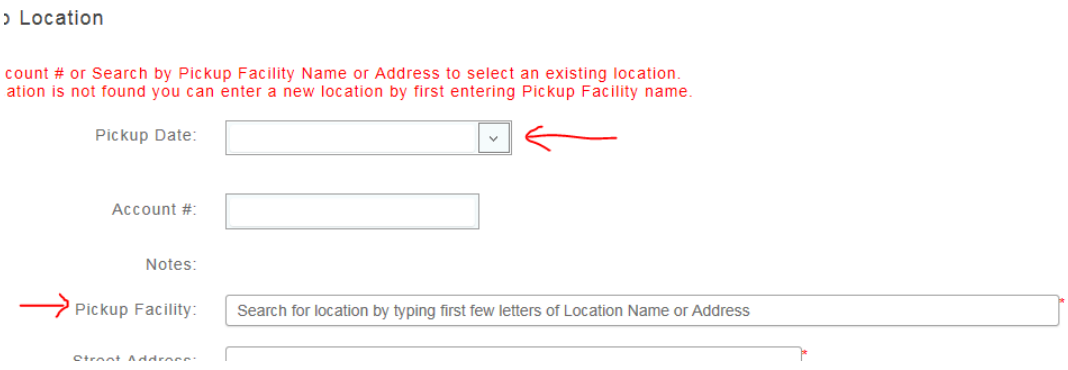
	e.g. 
13	On the Stat testing form, fill in • Patient's first and last name • Patient's DOB • Patient's MRN in the space marked "Registration #"
14	Call Flow Cytometry lab at 314-362-4628 to inform them that a sample is coming.
15	Proceed to next section.

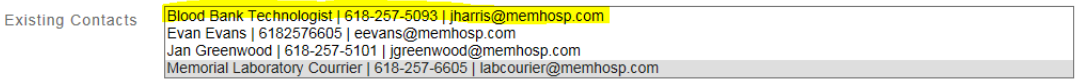
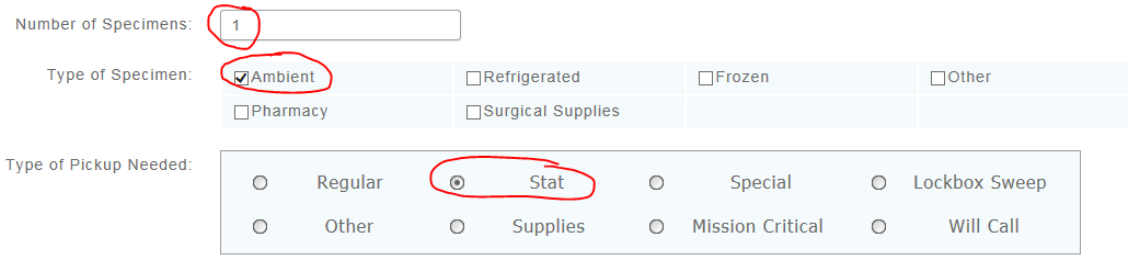
4. Packaging Sample

Step	Action
1	Place sample in a small biohazard bag.
2	Place the requisition with the Stat testing form attached in the front pocket of the bag.
3	Seal the pocket with a Barnes-Jewish lab shipping label. See *Appendix C.*
4	Place bag in area for Lab Express pickup.

5. Arranging for Courier Service

Step	Action
1	Open Internet Explorer
2	Type **www.labexp.com**
3	Click on **Sign In** in the top right corner. 

4	The user name is **memorial** and the password is **lab2@16.** Make sure it is for "Lab". 
5	Click **Enter Order** at the top of the screen. 
6	Click on the arrow to the right of the **Pickup Date** field and choose the date.
7	Place cursor in the **Pickup Facility** field and type "Belleville Memorial" and then wait for the menu to appear. 

8	Choose the Belleville Memorial Lab option as shown below. 
9	The address will populated automatically
10	In the **Pickup Instructions** field, type "Pick up at blood bank window".
11	In the **Existing Contacts** field, highlight the **Blood Bank Technologist** by clicking once on it. 
12	Enter the number of specimens that you are sending in the appropriate field.
13	Check the box next to **Ambient** for the temperature
14	Click the priority as **Stat** 
15	For the delivery location, type "Barnes" in the field and wait for the menu to pop up
16	Choose the "Barnes Jewish Lab" option that matches the address on the shipping label you put on the specimen.
17	In the **Pickup Instructions** field, type "Deliver to Flow Cytometry Department".
18	Click **Submit Order** at the bottom of the screen.
19	Lab Express should pick up specimen at blood bank window within 1 hour.

6. Receiving and Reporting of Results

Step	Action
1	The results should be called to the blood bank as well as faxed to the blood bank.
2	Once a hard copy of the results are obtained, go into the **Specimen** tab and then into **Enter**

	Results.
3	Enter the patient's information to retrieve the KB testing screen.
4	Enter the results from the Barnes report listed as **Fetal RBC Pct-Flow** in the **FETAL RBC FLOW** or **FETAL RBC PCT** field. (This will depend on whether the patient was Rh negative or Rh positive). See *Appendix D* for example of Barnes Report. • **NOTE: Result of < 0.05% MUST be entered as "0". MCARE will not calculate correctly if you put "<" in the field.**
5	If the patient is Rh negative, the result will look similar to this. 1 WHICH KB TEST? FLOW KB 2 PREVIOUS BT? YES 3 PAT'S RH TYPE? NEG 4 FETAL RBC PCT x 0.11 % 0.0-0.0 5 VIALS OF RHIG x 1 0-1
6	If the patient is Rh positive, the result will look similar to this. 13 PAT'S RH TYPE? POSITIVE 14 FETAL RBC FLOW x 1.51 15 FKB QUAL INTERP x LARGE
7	Press **F12** or click **Save**.

Interpretation

- **For FKB Qualitative Testing**

% Acid Resistant Cells	Results
<0.05	Negative
0.06-0.80	Small
0.81-1.49	Moderate
1.50 or Greater	Large

- **For FKB Quantitative**

% Acid Resistant Cells	Vials of RhIg
0.0-0.2	1
0.3-0.8	2
0.9-1.4	3
1.5-2.0	4
2.1-2.6	5
2.7-3.2	6
3.3-3.8	7
3.9-4.4	8
4.5-5.0	9
>5.1	See tech manual

Result Reporting

Step	Action
1	After testing has been verified, place faxed copy of report in bin at the front desk area to be scanned into the patient's chart in HIM.

References

AABB. *Standards for Blood Banks and Transfusion Services--29th Edition*. Std. 5.30.2, 5.30.3, 5.30.5. Bethesda, MD: American Association of Blood Banks; 2014

AABB. Perinatal Issues in Transfusion Practice. *Technical Manual--18th Edition*. Bethesda, MD: AABB; 2014. Pages 565-566

Related Documents

Appendix A: BJH Flow Cytometry Immuno Phenotyping Form
Appendix B: BJH Stat Testing Form
Appendix C: Barnes-Jewish Hospital Shipping Label
Appendix D: BJH Flow Cytometry Fetal Hemoglobin Report Example
Appendix E: Fetal Hemoglobin by Flow Cytometry Flowchart

Appendix A: BJH Flow Cytometry Immuno Phenotyping Form

Washington University in St. Louis
School of Medicine

FLOW CYTOMETRY IMMUNO PHENOTYPING
314-362-4628
St. Louis, Missouri 63110

Barnes-Jewish Hospital

COLLECTION INFORMATION ☐AM ☐PM
DATE _____ TIME _____ INITIALS _____

ACCOUNT INFORMATION
NAME **Memorial Hospital–Belleville**
ADDRESS **Laboratory–Sendouts**
4500 Memorial Drive
CITY **Belleville** STATE **IL** **62226**
PHONE **618–257–5093**
ORDERING PHYSICIAN
BILL TO: ☐ACCOUNT ☐PATIENT/INSURANCE ☐ALTERNATE
BLVMH
SEND ADDITIONAL COPY OF REPORT TO:
Blood Bank **618–257–6601**
CLIENT NUMBER/PHYSICIAN NAME PHONE/FAX NUM.
PHYSICIAN'S ADDRESS CITY, STATE, ZIP
BJH REGISTRATION #
REGISTERED BY

PATIENT
PATIENT'S NAME (LAST) (FIRST) (MI) SEX DATE OF BIRTH MO DAY YR PATIENT'S SS #
PATIENT'S ADDRESS CITY STATE ZIP PHONE
REFERENCE # DIAGNOSIS

RESP. PARTY
PATIENT'S RELATIONSHIP TO RESPONSIBLE PARTY ☐1-SELF ☐2-SPOUSE ☐3-CHILD ☐4-OTHER
NAME OF RESPONSIBLE PARTY (IF DIFFERENT FROM PATIENT) SOCIAL SECURITY (INSURED SS#)
ADDRESS OF RESPONSIBLE PARTY APT # DATE OF BIRTH MO DAY YR
CITY STATE ZIP
MEDICAID # STATE MEDICARE # (INCLUDE PREFIX/SUFFIX) ☐PRIMARY ☐SECONDARY MEDICARE RETIREMENT OR DISABILITY DATE

INSURANCE
INSURANCE COMPANY NAME PLAN CARRIER CODE
SUBSCRIBER / MEMBER # LOCATION GROUP #
INSURANCE ADDRESS PHYSICIAN'S PROVIDER #
CITY STATE ZIP
EMPLOYER'S NAME OR NUMBER WORKER'S COMP ☐YES ☐NO

CLINICAL HISTORY AND DIAGNOSIS:

SPECIMEN TYPE:

	Tube Type
X Peripheral Blood - Immune testing:	(LAV)
☐ Peripheral Blood Leuk/Lym workup:	LAV / 2 DK GRN
☐ Peripheral Blood PNH:	LAV / DK GRN
☐ Bone Marrow: _____	LAV / DK GRN / Core-Formalin
☐ Fluid: _____	FL
☐ Tissue: _____	
☐ FNA (Fine Needle Aspiration): _____	
☐ Other:	

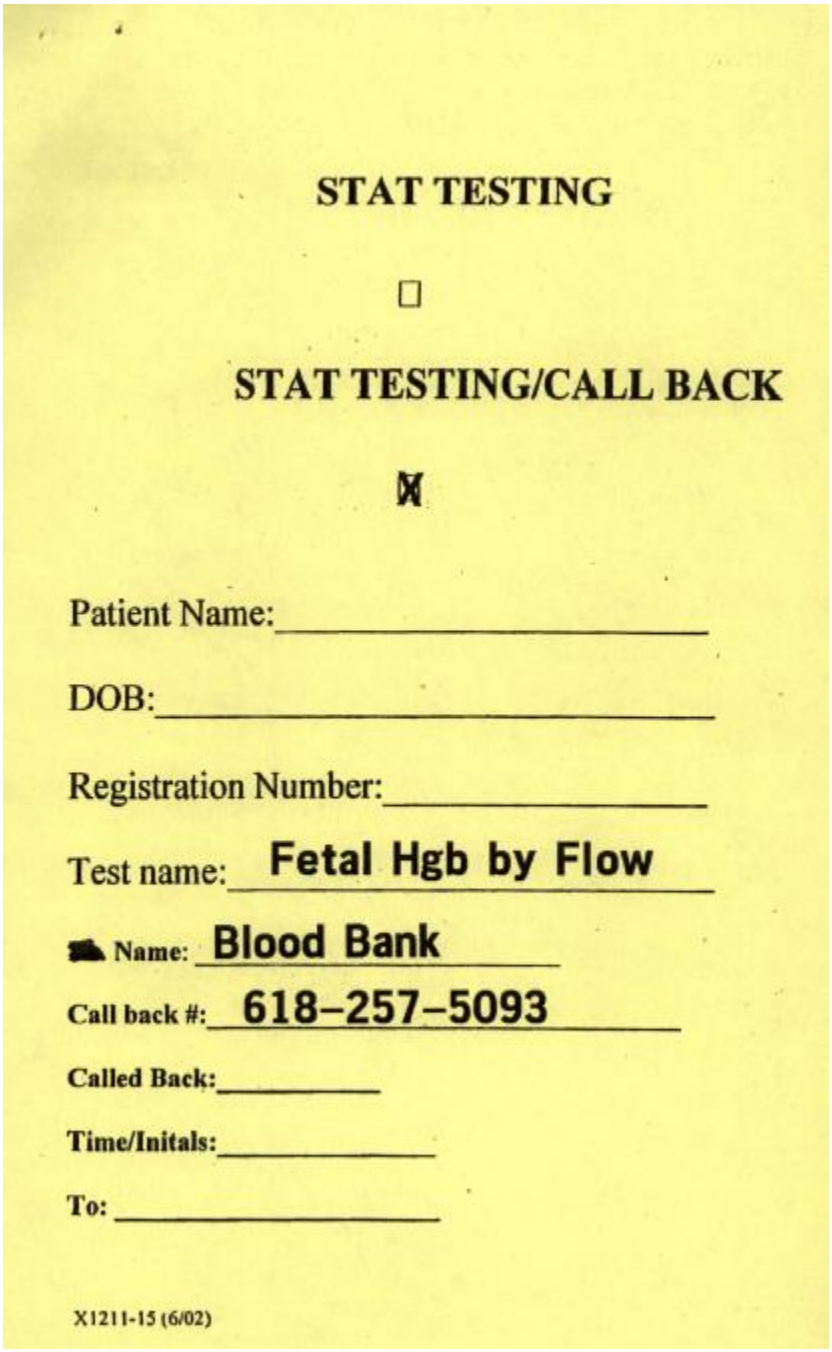
SAMPLE SUBMITTED:
COLLECTION TIME : AM PM COLLECTION DATE MO DAY YEAR

LABORATORY TEST
☐ Lymphoma WorkUp (Lymphoproliferative disorder ex: CLL, NHL, HCL)
☐ Leukemia WorkUp (Acute Leukemia ex AML, ALL, ANLL)
☐ PNH Profile Includes RBC-CD59, WBC-CD59 and FLAER
☐ Sezary Cell WorkUp
☐ Other (Please Specify) _____
X Other Flow Test: Fetal Red Blood Cell Percentage
☐ Lymphocyte Subpopulation 7 (CD3, CD4, CD8, CD19, CD16+56, CD2, HLA-DR)
☐ Lymphocyte Subpopulation 13 (CD3, CD4, CD8, CD19, CD16+56, CD16, CD2, CD40, HLA-DR, TCR)
☐ Immune Competence (CD3, CD4, CD8, CD19, CD16+56, CD4/CD8 Ratio)
☐ Immune Deficiency (CD4, CD8, CD4/CD8 Ratio)
☐ CD4
☐ CD45RA/CD45RO
☐ Adhesion Panel (CD11a, CD11b, CD11c, CD15, CD18)

CONTAINERS RECEIVED →	GRN DK GRN	FL FLUID	OT OTHER	COLLECTION TIME : AM PM	INITIALS

BJ 11-1195-2824 (02/07/11)

Appendix B: BJH Stat Testing Form



STAT TESTING

☐

STAT TESTING/CALL BACK

☒

Patient Name:_____

DOB:_____

Registration Number:_____

Test name: Fetal Hgb by Flow

Name: Blood Bank

Call back #: 618-257-5093

Called Back:_____

Time/Initals:_____

To: _____

X1211-15 (6/02)

Appendix C: Barnes-Jewish Hospital Shipping Label

Barnes-Jewish Hospital Lab

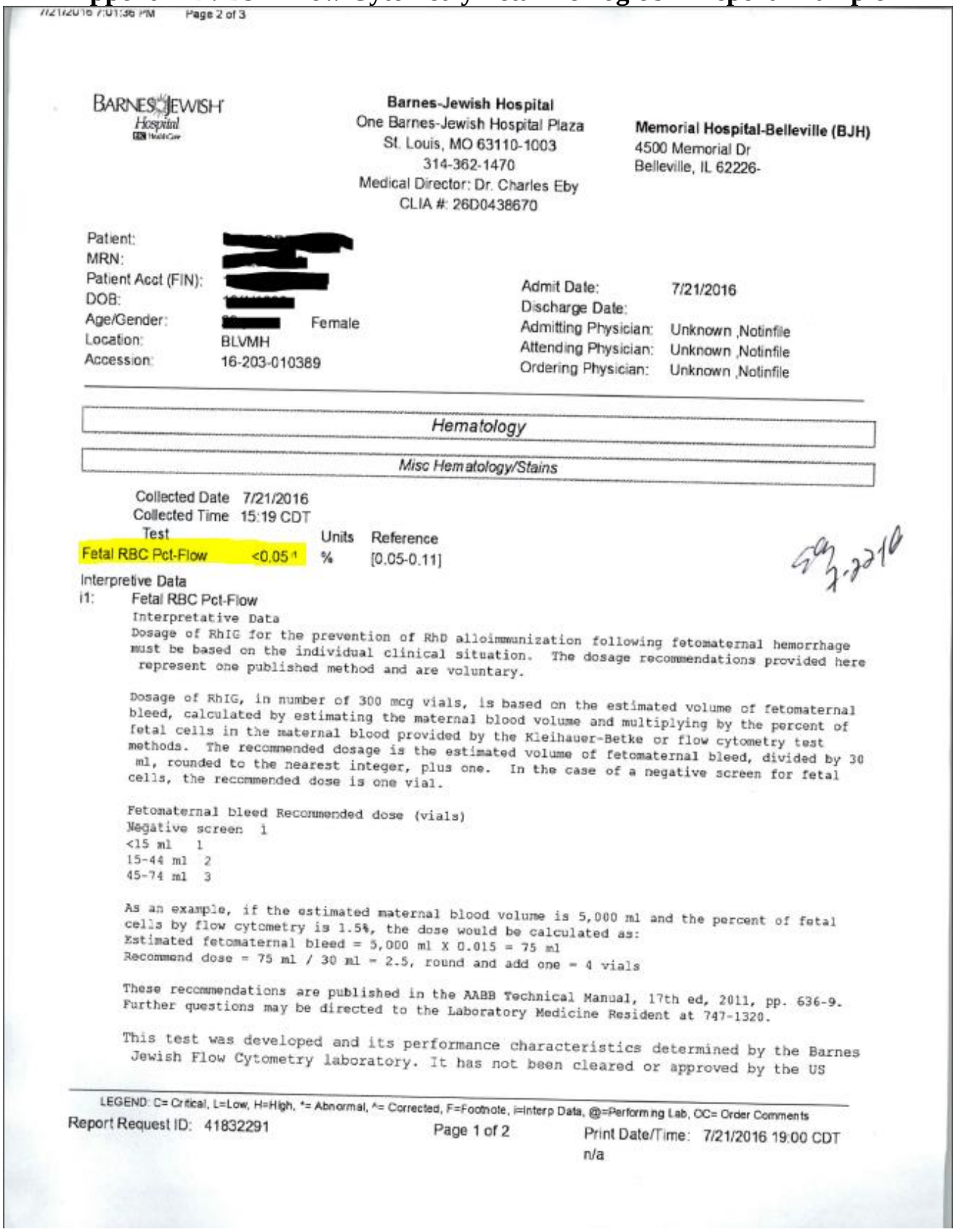
Flow Cytometry Department

425 Euclid Suite 4701

(4th Floor)

St. Louis, MO 63110

Appendix D: BJH Flow Cytometry Fetal Hemoglobin Report Example



7/21/2016 7:01:36 PM Page 2 of 3

BARNES-JEWISH Hospital

Barnes-Jewish Hospital
One Barnes-Jewish Hospital Plaza
St. Louis, MO 63110-1003
314-362-1470
Medical Director: Dr. Charles Eby
CLIA #: 26D0438670

Memorial Hospital-Belleville (BJH)
4500 Memorial Dr
Belleville, IL 62226-

Patient: [redacted]
MRN: [redacted]
Patient Acct (FIN): [redacted]
DOB: [redacted]
Age/Gender: [redacted] Female
Location: BLVMH
Accession: 16-203-010389

Admit Date: 7/21/2016
Discharge Date:
Admitting Physician: Unknown ,Notinfile
Attending Physician: Unknown ,Notinfile
Ordering Physician: Unknown ,Notinfile

Hematology

Misc Hematology/Stains

Collected Date 7/21/2016
Collected Time 15:19 CDT

Test		Units	Reference
Fetal RBC Pct-Flow	<0.05 *	%	[0.05-0.11]

7-22-16

Interpretive Data
i1: Fetal RBC Pct-Flow
Interpretative Data
Dosage of RhIG for the prevention of RhD alloimmunization following fetomaternal hemorrhage must be based on the individual clinical situation. The dosage recommendations provided here represent one published method and are voluntary.

Dosage of RhIG, in number of 300 mcg vials, is based on the estimated volume of fetomaternal bleed, calculated by estimating the maternal blood volume and multiplying by the percent of fetal cells in the maternal blood provided by the Kleihauer-Betke or flow cytometry test methods. The recommended dosage is the estimated volume of fetomaternal bleed, divided by 30 ml, rounded to the nearest integer, plus one. In the case of a negative screen for fetal cells, the recommended dose is one vial.

Fetomaternal bleed Recommended dose (vials)
Negative screen 1
<15 ml 1
15-44 ml 2
45-74 ml 3

As an example, if the estimated maternal blood volume is 5,000 ml and the percent of fetal cells by flow cytometry is 1.5%, the dose would be calculated as:
Estimated fetomaternal bleed = 5,000 ml X 0.015 = 75 ml
Recommend dose = 75 ml / 30 ml = 2.5, round and add one = 4 vials

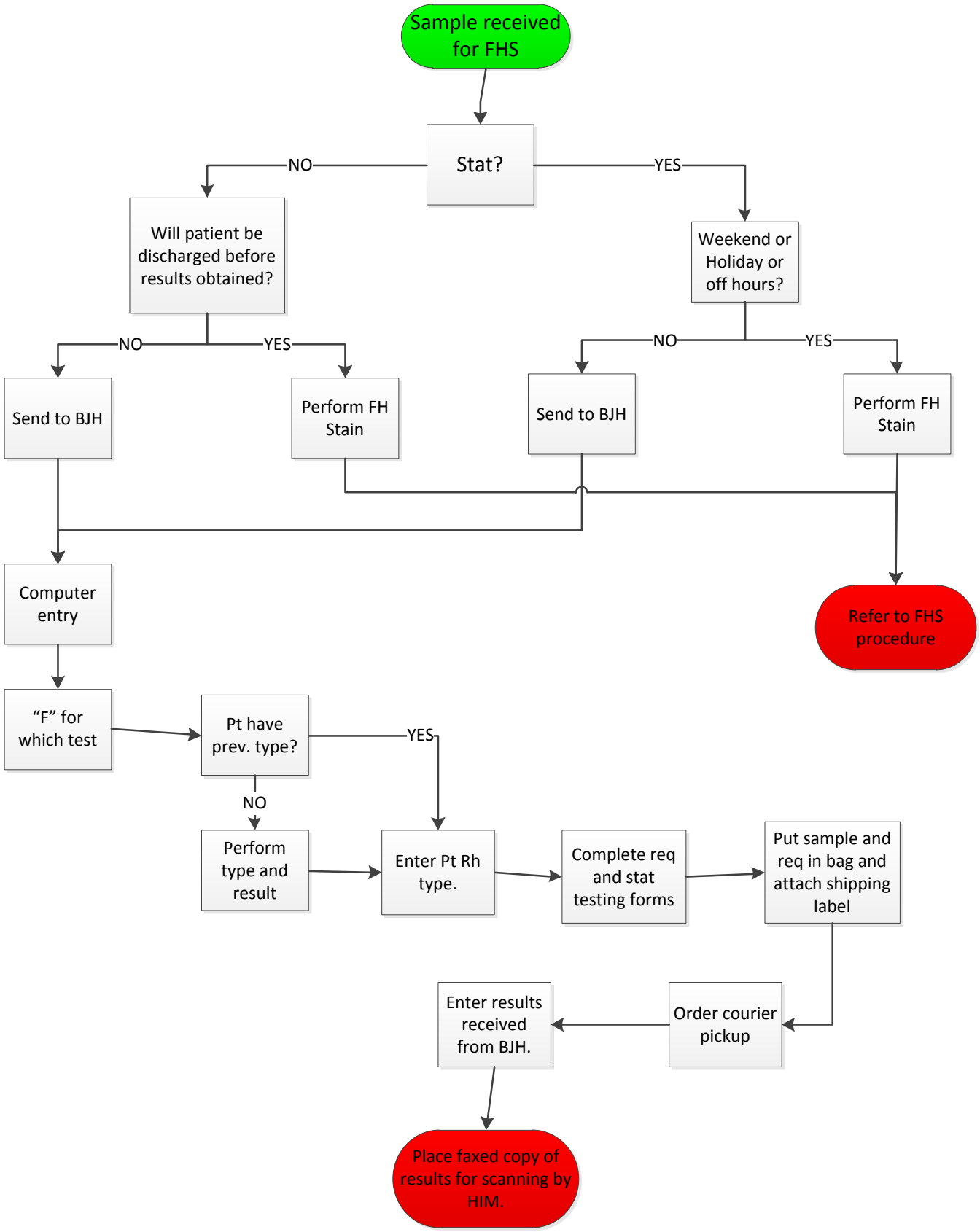
These recommendations are published in the AABB Technical Manual, 17th ed, 2011, pp. 636-9. Further questions may be directed to the Laboratory Medicine Resident at 747-1320.

This test was developed and its performance characteristics determined by the Barnes Jewish Flow Cytometry laboratory. It has not been cleared or approved by the US

LEGEND: C= Critical, L=Low, H=High, *= Abnormal, ^= Corrected, F=Footnote, i=Interp Data, @=Performing Lab, OC= Order Comments

Report Request ID: 41832291 Page 1 of 2 Print Date/Time: 7/21/2016 19:00 CDT
n/a

Appendix E: Fetal Hemoglobin by Flow Cytometry Flowchart



PROCEDURE AND FORM CHANGE CONTROL

Title: Fetal Hemoglobin by Flow Cytometry										
Written		**Validated**		**Path Review**		**Review**		**Effective**		**Reason for Revision**
Date	**By**	**Date**	**By**	**Date**	**By**	**Date**	**By**	**Date**	**By**	
10/4/2016	**JLH**	**10/5/16**	**GJM**	**10/14/16**	**ESB**			**10/21/16**	**JLH**	
Revised										

Location of any copy(s) of the procedure:

Out of use:

Date:_____By:_____Reason:_____