TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	10/18/22
Department:	Core Lab	**Due Date:**	11/1/22

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Title: Kleihauer-Betke (KBT) Sure-Tech Kit for Fetal RBC Identification (AHC.HG 1022)
Description of change(s):
New KBT Method SOP acknowledgement This revised SOP was implemented September 5, 2022

Document your compliance with this training update by taking the quiz in the MTS system.

AHC.HG 1022 Kleihauer-Betke (KBT) Sure-Tech Kit for Fetal RBC Identification

Copy of version 2.0 (approved and current)

Last Approval or Periodic Review Completed	9/5/2022
Next Periodic Review Needed On or Before	9/5/2024
Effective Date	9/5/2022

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Organization Adventist HealthCare

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	9/5/2022	2.0	Nicolas Cacciabeve	
Approval	Core lab approvals	9/1/2022	2.0	Robert SanLuis Robert SanLuis	
Approval	Lab Director	8/29/2022	1.0	Nicolas Cacciabeve	
Approval	Core lab approvals	8/25/2022	1.0	Robert SanLuis Robert SanLuis	

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
2.0	Approved and Current	Major revision	8/31/2022	9/5/2022	Indefinite
1.0	Retired	Initial version	8/25/2022	8/29/2022	9/5/2022

Technical SOP

Title	Kleihauer-Betke (KBT) Sure-Tech Kit for Fetal RBC Identification	
Prepared by	Ashkan Chini	Date: 8/25/22
Owner	Robert SanLuis	Date: 8/25/22

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Kleihauer-Betke	Fetal Hemoglobin-Differential Staining Kit	KBT

Synonyms/Abbreviations
KBT, Fetal Cell Screen

Department
Hematology

2. ANALYTICAL PRINCIPLE

Fetal hemoglobin is both alkali and acid resistant. Consequently, it is not eluted from fresh blood slides fixed in an ethanol/phosphate buffer. After this treatment, cells containing fetal hemoglobin stain with eosin, while those cells without any Hb F appear as colorless ghosts.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Maternal blood should be collected as soon as possible following delivery or sensitizing event. Collect the sample in EDTA. Do not use cord blood.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred	Whole Blood EDTA	
-Other Acceptable	None	
Collection Container	Lavender top tube	
Volume - Optimum	5.0 mL	
- Minimum	1.0 mL	
Transport Container and Temperature	Collection container at room temperature	
Stability & Storage Requirements	Room Temperature:	Unacceptable
	Refrigerated:	14 days (2-8°C)
	Frozen:	Unacceptable

Criteria	
Timing Considerations	Upon receipt in the department, samples should be stored refrigerated until tested.
Unacceptable Specimens & Actions to Take	Cord blood or serum samples, Clotted samples, and specimens collected after RhIg administration. Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request redraw. Credit the test with the appropriate LIS English text code. Lipemia and Icterus samples are acceptable.
Other Considerations	None

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

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 as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Fetal Hemoglobin Kit	Sure-Tech Diagnostics, Inc. Cat. no. ST-101 A – C
0.85% Saline	Fisher Healthcare, Cat. no. 23 535 435

4.2 Reagent Preparation and Storage

Reagent Kit	Fetal Hemoglobin
Reagent 1	Red Cell Fixing Solution, 80% Ethanol
Reagent 2	Citrate/Phosphate Buffer, Preservative
Reagent 3	Hemoglobin Staining Solution, 0.1% Erythrosin Stabilizer
Container	Individual Solution Bottles
Storage	Store at 20-25°C
Stability	Both opened and unopened reagents remain stable until expiration date stamped on reagent bottle.

Preparation	Reagents are supplied ready for use. No additional preparation is required.
Other Considerations	Reagents may be reused until deterioration in the quality of the slides are noted. Do not pour used reagent back into original containers.

Reagent	0.85% Saline
Preparation	Reagent is ready for use.
Storage/Stability	• Unopened reagent remains stable until the expiration date printed on the container. • Opened reagent remains stable for 30 days. • Reagent that is poured from the original container into a smaller container for staining purposes only remains stable for 24 hours.

5. CALIBRATORS/STANDARDS

None

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Positive and Negative	Obtained from in house patients or specimens

6.2 Control Preparation and Storage

Control	Positive Control
Preparation	Obtain a fresh cord blood specimen located in Blood Bank. Place 2 drops of the cord blood and 2 drops of a male patient's blood (fresh EDTA sample preferred) in a 12 x 75mm glass tube. Mix and prepare two thin smears and let them air dry.
Storage/Stability	Slides are routinely prepared with each patient test performed, but are stable for 2 weeks when stored in the freezer at -20C or colder.

Control	Negative Control
Preparation	Use a male patient's fresh EDTA specimen and prepare two thin blood smears. Let them air dry.
Storage/Stability	Slides are routinely prepared with each patient test performed, but are stable for 2 weeks when stored in the freezer at -20C or colder.

6.3 Frequency

Both positive and negative controls are performed with each patient testing.

6.4 Tolerance Limits and Criteria for Acceptable QC

IF the QC result is …	THEN…
not reacting as expected (see section 10.1 for explanation)	repeat using new cord and male patient's blood; and fresh stain

- All corrective action must be documented as outlined in the Laboratory Quality Control Program.
- If repeating test does not produce acceptable QC, notify the supervisor immediately.
- No patient results are to be reported until acceptable QC results are obtained.

6.5 Documentation

- Quality Control is documented on the Kleihauer Betke Quality Control Log.
- Quality control records are reviewed daily at the bench, weekly by the Lead Technologist or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each KBT test or batch of KBT tests must be tested with positive and negative control materials.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Consult the Laboratory QC program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

None

7.2 Equipment

- Microscope
- Timer

7.3 Supplies

- Glass slides
- 12 x 75 mm glass tubes

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.

8.1	Instrument Set-Up Protocol
1.	This procedure must be performed at room temperature (20-25ºC)
2.	Mix the blood sample by gentle inversion.
3.	Place 3 drops of 0.85% saline and 2 drops of blood into a glass test tube, mix gently.
4.	Place one drop of diluted blood on a glass slide near one end. Prepare a smear by drawing the edge of another slide through the drop and across the slide.
5.	Air dry the slide at room temperature.
6.	Place the slide in the jar containing sufficient Red Cell Fixing Solution to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the fixing solution and allow the slide to remain in the solution for 5 minutes.
7.	Remove the slide from the fixing solution, rinse thoroughly with deionized water and air dry.
8.	Place the dry slide in the jar containing sufficient Citrate/Phosphate Buffer to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the buffer and allow the slide to remain in the solution for 10 minutes.
9.	Remove the slide from the buffer solution and blot excess buffer from the slide.
10.	Place the wet slide in the jar containing sufficient Hemoglobin Staining Solution to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the stain and allow the slide to remain in the stain for 3 minutes.
11.	Remove the slide from the Hemoglobin Stain Solution, rinse thoroughly with deionized water and allow to dry at room temperature.
12.	Slides must be examined by using oil immersion. Fetal cells will stain a dark reddish-pink while adult cells will appear white to light pink with a slightly darker center. Other cells may also stain to a varying degree and these cells must be identified so as not to be counted as fetal cells.
13.	Examine smears as specified in section 10.1

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

$$\% \text{ fetal cells} = \frac{\text{Total fetal RBCs}}{\text{Total adult RBCs}} \times 100$$

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

The smears should be read as soon as possible after drying. This test is considered a STAT test and reporting a positive fetal bleed as soon as possible is paramount. A 100X microscope should be used for optimal cellular contrast and counting. Scan for the area of the slide with approximately 200-300 maternal cells touching but not stacked with the aid of a 40X ocular.

1. Scan the stained control slides at 400X magnification to determine the presence of fetal and non-fetal cells. The positive control should have both dark staining fetal and ghost non-fetal cells present. The negative should not have any of the fetal cells there; should only be ghost cells present. Record the results of the controls and patient(s) on the worksheet and place in the appropriate binder.
2. Scan the stained patient smear at 400X magnification to determine the area in which an even, but not scanty, distribution of cells is seen. **Count at 1000X magnification**.
3. Within this area, begin reading for a total of ten consecutive fields (*do not view the smear through the microscope to select the fields, for this may skew the count*).
4. Count the number of fetal erythrocytes and the number of adult erythrocytes in each field. Record the count of fetal erythrocytes and the number of adult erythrocytes in each field on the Kleihauer Betke Quality Control Log.
5. Total the number of fetal red blood cells observed in the ten fields.
6. Total the number of adult red blood cells observed in the same ten fields.
7. Calculate the ratio of fetal red blood cells to adult red blood cells by dividing the total number of fetal red blood cells observed in the ten microscope fields by the total number of adult red blood cells observed in the same ten microscope fields. Then multiply the ratio by 100 to get percentage.

Example:

Microscope field	Fetal Cells	Adult cells
1	2	245
2	1	247
3	0	242
4	1	252
5	2	253

6	1	245
7	2	262
8	1	262
9	1	200
10	0	220
Total cells counted	11	2408

Cell ratio = 11÷ 2408 = 0.0045
0.0045 x 100 = 0.45 %

10.2 Determining RhIG Dosage

Sunquest will automatically calculate the recommended number of vials of RhIG to give to Rh-negative (including Rh intdeterminate) or weak D positive patients. RhIG is not indicated for Rh-positive patients. If the Rh type is entered as "positive," Sunquest will automatically recommend 0 vials of RhIG.

% Fetal Cells	RhIG Vials to Inject
<0.3	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 – 5.6	10
5.7 – 6.2	11
6.3 – 6.8	12
6.9 – 7.4	13
7.5 – 8.0	14
8.1 – 8.6	15

10.3 Rounding

N/A

10.4 Units of Measure

%

10.5 Clinically Reportable Range (CRR)

N/A

10.6 Review Patient Data

Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of positive results). Resolve any problems noted before issuing patient reports.

10.7 Repeat Criteria and Resulting

Reporting Results:
All reports are entered into the computer system via manual entry.
Function: MEM
Worksheet: SHE1

IF the result is …	THEN…
No fetal cells are detected	Result using English Text Code - **NFET**
Fetal cells seen	Result the percent to 2 decimals places as calculated in section 10.1

Notify the patient care area if testing results indicate the patient is Rh-negative or weak D positive **AND** requires more than 1 vial of RhIG.

11. EXPECTED VALUES

11.1 Reference Ranges

No fetal cells detected

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

The cytological detection of cells containing fetal hemoglobin is of importance in determining the distribution of fetal hemoglobin in red cells. It is also useful in determining the presence of fetal red blood cells in the maternal circulation, which assesses the magnitude of fetal maternal hemorrhage and enables calculation of the dosage of Rh immune globulin to be given.

13. PROCEDURE NOTES

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

- Perform test immediately after delivery to minimize effect of ABO incompatibility.
- Timing is critical in fixing, staining and eluting the smears.
- Oil immersion lens must not be used to *scan* for the proper counting area.
- When counting, do not use a bright microscope light to increase the contrast between adult and fetal cells.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

Maternal antibody reactive with fetal A and B antigens may remove many of the fetal cells

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Laboratory Safety Manual
3. Safety Data Sheets (SDS)
4. Quest Diagnostics Records Management Procedure
5. Specimen Acceptability Requirements (Lab policy)
6. Repeat Testing Requirement (Lab policy)
7. Kleihauer Betke Quality Control Log (AG.F116)
8. Current package insert from Sure-Tech Diagnostics, Inc.

17. REFERENCES

1. Sure-Tech Diagnostic Assoc., Inc., Kleihauer-Betke Fetal Hemoglobin, revised 10/2017

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	8/31/22	Title	Changed Title	D Collier	R SanLuis

19. ADDENDA

None