Sutter Health
Sutter Roseville Medical Center

Origination:	*7/30/2019*
Effective:	*Upon Approval*
Final Approved:	*N/A*
Last Revised:	*2/24/2020*
Next Review:	*2 years after approval*
Owner:	*Alex Alba: Spvr, Laboratory*
Policy Area:	*Lab - Serology*
References:	
Applicability:	*Sutter Roseville Medical Center*

Performing a Fecal Lactoferrin Test, IM.ANA10.10-/-RV.XX

Performing a Fecal Lactoferrin Test

Purpose	The *LEUKO EZ VUE®* test is an immunochromatographic test for the qualitative detection of elevated levels of fecal lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The *LEUKO EZ VUE®* test detects lactoferrin in liquid, semi-solid, and solid fecal specimens. A positive test result indicates an increased level of fecal lactoferrin and warrants additional testing.
Policy	• This procedure is to be followed when performing fecal lactoferrin testing using the *LEUKO EZ VUE®* test kit. ◦ Test code: **LACFS** (*Lactoferrin, Stool*)
Equipment, Reagents and Supplies	• Timer • Vortex • Disposable Pipettes or MLA Pipette • *LEUKO EZ VUE®* test kit
Reagent, Storage and Stability	• All reagents included in this kit should be stored refrigerated or at room temperature, 2-30 °C, until the expiration dates printed on reagent labels. ◦ **Do not freeze** reagents. ◦ Reagents from different kits should not be mixed. ◦ Do not use the kit past the expiration date. • Membrane cassettes should be kept in the sealed pouches until used. • Keep the membrane cassettes dry before use.
Specimen Requirements	• Collect fecal specimen into a clean, airtight container with no preservatives. • Specimens that are in transport media or that have been preserved in 10% formalin, Merthiolate Formalin, Sodium Acetate Formalin, Polyvinyl Alcohol, or other fixatives **cannot** be used and will be **rejected.** • Specimens may be stored between 2-8ºC, or at room temperature for up to **2 weeks** from time of collection. • If samples need to be stored longer than 2 weeks, they may be stored frozen at -20ºC or lower. • Diluted specimens may be stored between 2-8ºC or at room temperature for up to **48 hours** after testing and then must be discarded.
Safety Precautions	• Patient samples, controls, and test devices should be handled as though they could transmit disease. • Always wear gloves when performing the test and when handling reagents in this kit as some reagents are preserved with 0.095% (w/w) sodium azide. • The *Positive Control* contains lactoferrin, which is a human derived material. Material has been tested and found negative for antibody to HIV-1, HIV-2, HCV, and HbsAg. No known test method can offer complete assurance that infectious agents are absent.
Quality Control	**Internal controls:** *Internal controls are contained within the membrane cassette and therefore are evaluated with each test.* • A red band appearing at the 'C' side of the results window serves as a positive internal control and indicates the test has been performed correctly, that proper flow occurred and that the test reagents were active at the time of use. • A clear background in the result area serves as a negative internal control. If the test has been performed correctly and reagents are working properly, the background will be clear to give a discernible result. • Internal QC is documented on *Manual Test Patient log.*

External controls: *External controls are to be run **monthly** and with each new lot and/or shipment. New lot numbers and/or shipments are also to be checked against old external controls before being put into use for patient testing.*

- External QC is **not** to be diluted like patient samples prior to testing. Refer to "Procedure-Test Assay" section for instructions.
- The results expected with the controls are described in the "Interpretation of Results" section.
- External controls are used to monitor reagent reactivity and test performance.
- Failure of external controls to produce expected results suggests the test was not performed correctly (i.e., incorrect volume of reagents added, incorrect incubation temperature or times used, or that reagents were not brought to room temperature prior to testing).
- Repeat control tests as first step in determining root cause of failure.
- The kit should not be used for patient testing if the controls do not produce the expected results.
- External QC is documented on *Immunology QC log: Fecal Lactoferrin QC.*

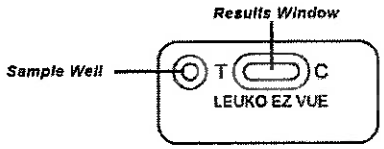
Procedure ***Specimen Preparation***

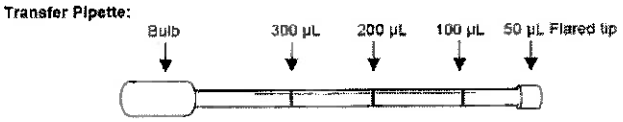
Follow the steps below to prepare diluted fecal specimens for testing.

Step	Action
1.	Bring all reagents and only the required number of membrane cassettes to room temperature prior to testing.
2.	Verify specimen(s) and corresponding patient barcode label(s) are accounted for and acceptable for testing.
3.	Determine if stool sample is for an infant less than 1 year old. ***NOTE:** A disclaimer must be entered when testing infants. Refer to Reporting Results section.*
4.	Setup and label a plastic dilution tube for each specimen to be tested.
5.	Add 2.5mL of *Diluent* to each dilution tube.

If	Then
Specimen is liquid	Use a transfer pipette to add 50 µL (flared section) of liquid fecal specimen to the corresponding tube.
Specimen is formed/solid	Use a transfer pipette to add 0.05 g (flared section) of fecal specimen and add to the corresponding tube.

6.	Place a filter tip onto the top of each tube containing diluted sample and insert the tip firmly. • **NOTE**: This represents a 1:50 dilution of the specimen.
7.	Vortex the tubes for 10 seconds and ensure specimen is well mixed before performing the test. • **NOTE**: If not performing test after preparing the diluted specimen, store tube(s) between 2° and 8°C or room temperature, until test is performed.





Procedure *Test Assay*

Follow the steps below to perform a qualitative fecal lactoferrin test.

Step	Action
1.	Remove required number of membrane cassettes, one per specimen, from the foil bags.
2.	Place membrane cassettes on work surface with the results window facing upwards and label cassettes accordingly. Avoid contact with the membrane located in the Results Window.
3.	• **Diluted specimen**: Holding each diluted specimen tube vertically, dispense **5 drops** (150 µL) into the sample well of a membrane cassettes.

- **NOTE**: Normal fecal specimens contain low levels of lactoferrin and the dilutions recommended in the kit are designed to detect an increase in lactoferrin over background levels.
- **External QC**: Add **3 drops** (150 µL) of *Positive Control* or 150 µL of *Diluent* using the transfer pipette into the sample well of the cassette.
  - **NOTE**: *Diluent* is used as the Negative External QC and Positive External QC is supplied with the kit

4.	Incubate each membrane cassette for 10 minutes at room temp.
5.	Read results **promptly** at 10 minutes and look for the appearance of a red line at the "C" control portion and/or "T" test portion of the window. • **NOTE**: The red line may appear faint to dark in color.
6.	Proceed to "Interpretation of Results" section.

Interpretation of Results

Follow the steps below to interpret the results of the fecal lactoferrin test.

Step	Action
1.	Follow the steps below to interpret the results of the fecal lactoferrin test.

Observation	Result
The presence of a single red line at "T" test portion and a single red line at "C" control portion *(regardless of the intensity)*	**Positive - Presence** of elevated fecal lactoferrin and a properly reactive control
The presence of a single red line at "C" control portion only. No line should be visible at "T" test portion.	**Negative - Absence** of elevated fecal lactoferrin and a properly reactive control
No presence of a single red line at "C" control portion or if no lines appear on the completed membrane cassette.	**Invalid** results. Test should be repeated.

2.	Record the patient and internal QC results on the Manual Test Patient log.

Positive Test Result

Negative Test Result

Invalid Test Result

Invalid Test Result

Reporting Results

Follow the steps below to enter results in Sunquest.

Step	Action
1.	Enter results in Sunquest, using function MEM and worksheet, **RVLACF**.
2.	Enter Accession number to be resulted at the Accession number prompt.

If	Then report
Absence of elevated fecal lactoferrin	NEG • *Negative*
Presence of elevated fecal lactoferrin	POS • *Positive*
Invalid results after repeat testing	INVALC • *Result invalid. Suggest repeat testing if clinically indicated.*
Patient is an infant (<1	**Append free text to result:** "Lactoferrin is present in increased levels in

		year old) and result is POSITIVE	breast milk. Results should be interpreted with caution in conjunction with breast feeding status".
	3.	Enter 'A' to accept results.	
	4.	Verify correct manual entry of results and document on the Manual Test Patient log (under RVS column)	

Technical Limitations	• The *LEUKO EZ VUE®* test detects elevated levels of lactoferrin released from fecal leukocytes as a marker of intestinal inflammation. The test may not be appropriate in immunocompromised persons • The 1:50 dilution of fecal specimen recommended in the brochure has been evaluated in clinical trials and found to be optimal for fecal dilutions. The use of lower dilutions may result in positive reactions due to the presence of normal lactoferrin levels. Therefore, only the dilution recommended in the brochure should be used. • At this time, the *LEUKO EZ VUE®* test has not been clinically evaluated for detecting leukocytes in other types of clinical specimens. • The intensity of a positive sample test line does not indicate the amount of lactoferrin or severity of disease. • Fecal samples from breast fed infants should not be used with this assay. A false positive result may be caused due to the presence of lactoferrin in breast milk. For non-breast fed infants, results should be interpreted normally. • Lactoferrin levels in breast milk remain high over time and any breast feeding runs the risk of a false-positive result on this assay
Related Documents	• Immunology Quality Control log • Manual Test Patient log
References	• *LEUKO EZ VUE®* product insert, Alere, Issued:07/2016, RMS#: 91-355-01

All revision dates: 2/24/2020, 7/30/2019

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
Laboratory Director	Lindsey Westerbeck: Dir, Lab	pending