Special Hematology/ 102 LEUKO EZ - FECAL LACTOFERRIN

Copy of version 1.0 (approved and current)

Last Approval or Periodic Review Completed 4/2/2024

Next Periodic Review Needed On or Before 4/2/2026

Effective Date 8/15/2021

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Printed By Taneisha Wallace

Organization Howard University Hospital

Comments for version 1.0
Initial version

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Periodic review	Lab Director	4/2/2024	1.0	Ali Mousa Ramadan MD	
Periodic review	Medical Pathologist	3/22/2024	1.0	Lekidule Taddesse-Heath	
Approval	Lab Director	8/15/2021	1.0	Ali Mousa Ramadan	
Approval	Laboratory Operations Manager	8/13/2021	1.0	Wendell McMillan	
Approval	Quality Coordinator	8/1/2021	1.0	Lorraine Foster	Initial electronic version

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.0	Approved and Current	Initial version	7/22/2021	8/15/2021	Indefinite

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HOWARD UNIVERSITY HOSPITAL

DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

STANDARD OPERATING POLICY AND PROCEDURE MANUAL

Special Hematology/102 Leuko EZ- Fecal Lactoferrin

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.

The LEUKO EZ VUE® overcomes the problems of microscopy by utilizing immuno-chromatography technology and provides results in 10 minutes. The assay detects elevated levels of lactoferrin in fecal samples. Lactoferrin is very stable and is not degraded during infections by the toxins of pathogens such as C. difficile (6).

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PRINCIPLE OF THE TEST

The LEUKO EZ VUE® test utilizes rabbit anti-lactoferrin antibodies that are conjugated directly to gold particles. The Membrane Cassette contains two stripes of immobilized antibodies. One stripe contains anti-lactoferrin antibodies. The other, representing a control stripe, contains anti-IgG antibodies. The diluted sample and gold conjugate migrate by capillary action when the sample is added to the well. If elevated lactoferrin is present in the sample, gold conjugate-lactoferrin complexes form and are captured by the immobilized anti-lactoferrin antibodies in the stripe. The lactoferrin-conjugate-antibody complexes appear as a single red line in the test portion of the Results Window. In the control stripe, conjugate binds to the immobilized anti-IgG antibodies, demonstrating correct migration of the sample and conjugate along the membrane. The conjugate-anti-IgG antibodies appear as a single red line in the control portion of the Results Window.

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REAGENTS

- Diluent, 65 mL (Ready-to-use, contains phosphate-buffered saline, detergent and 0.05% ProClin® 300)
- Membrane Cassettes, 25 (1 Membrane Cassette per pouch; each membrane is coated with anti-lactoferrin antibodies and contains antibodies conjugated to colloidal gold)
- Positive Control, 3.5 mL (phosphate-buffered saline containing purified hu- man lactoferrin)
- Disposable plastic pipettes, 25 (flared section = 50 µL)
- Disposable sample preparation devices, 25 (25 tubes and 25 filter tips)

PRECAUTIONS

1. for in vitro diagnostic use only.

2. Reagents from the kit box should be at room temperature before use.

3. The pouch containing Membrane Cassette should be opened just before use.

4. Keep the Membrane Cassettes dry before use.

5. Reagents from different kits should not be mixed. Do not use the kit past the expiration date.

6. Use the dilution of fecal specimen as recommended in the kit. 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.

7. Do not freeze the reagents. The kit should be stored between 2° and 30°C.

8. All Membrane Cassettes must be read promptly at 10 minutes.

9. To minimize the effects of static electricity, place all Membrane Cassettes with Results Window facing upwards on damp paper towels.

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10. 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.

11. 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. All human source products should be handled as potentially infectious material. A procedure for handling biohazards is published in the CDC/NIH Manual of Biosafety in Microbiology & Biomedical Laboratories.

12. Specimens and Membrane Cassettes should be handled and disposed of as potential biohazards after use.

13. Wear disposable gloves when doing the test.

14. The Diluent reagent contains 0.05% ProClin® 300 as a preservative. Although the concentration is low, ProClin® 300 is known to be harmful. If skin irritation or rash occurs, get medical advice/attention. Take off contaminated clothing and wash it before reuse. Handle reagents according to existing regulations for laboratory safety and good laboratory practice. Safety Data Sheets for this product are available upon request, contact technical support.

15. Follow your national, regional, and local ordinances accordingly for waste disposal regulations.

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PRELIMINARY PREPARATIONS

1. All reagents must be removed from the kit box and allowed to reach room temperature prior to use in the assay.

2. Membrane Cassette preparation. Each pouch contains 1 Membrane Cassette coated with polyclonal antibody specific for lactoferrin. Each specimen or control will require one of these Membrane Cassettes. Avoid contact with the membrane located in the result window

COLLECTION AND HANDLING OF FECAL SPECIMENS

- Collect fecal specimens into a clean, airtight container with no preservatives.
- Specimens should be stored between 2º and 8ºC or room temperature for up to 2 weeks from time of collection then stored frozen at -20ºC or lower.
- Diluted specimens should be stored between 2º and 8ºC or at room temperature for up to 48 hours then discarded. Mix (vortex) specimens thoroughly prior to performing the assay. This includes complete mixing of the specimen prior to transfer to Diluent as well as complete mixing of the diluted specimen prior to performing the assay.
- 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.

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Prepare Diluted Specimen:

Fecal Specimens:

- Set up a single plastic tube for each specimen to be tested.
- For each specimen, add 2.5 mL of Diluent to a dilution tube. Use a transfer pipette to add 50 µL (flared section) of liquid fecal specimen.
- For formed/solid fecal specimens, use a transfer pipette to add 0.05 g (flared section) or weigh 0.05 g of fecal specimen and add to the tube containing Diluent.
- Next, place a filter tip onto the top of the tube containing diluted sample and insert the tip firmly. This represents a 1:50 dilution of the specimen.

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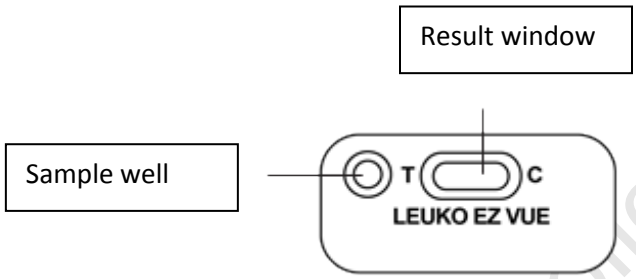
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

STANDARD OPERATING POLICY AND PROCEDURE MANUAL

- Vortex the tubes for 10 seconds and store between 2° and 8°C until the test is per- formed.
- Vortex again before transferring 5 drops of diluted specimen to Sample Well indicated in the diagram of the Membrane Cassette.

Membrane Cassette Diagram



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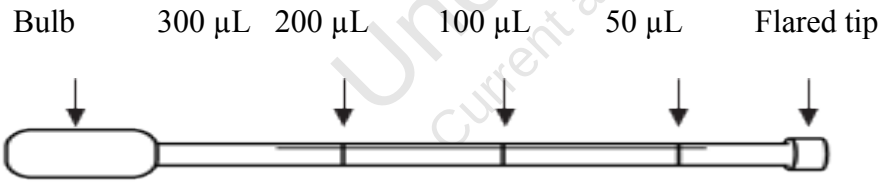
Transfer Pipette:



PROCEDURE

1. Obtain Membrane Cassettes. Remove required number of Membrane Cassettes, one per specimen, from the foil bags.

2. Place Membrane Cassettes on damp paper towels with the Results Window facing upwards and label cassettes accordingly.

3. Holding each diluted specimen tube vertically, dispense 5 drops (150 µL) into the Sample Well of a Membrane Cassette. If running 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).

4. Incubate each Membrane Cassette for 10 minutes at room temperature.

5. Read results promptly at 10 minutes: Observe the Results Window of each completed

Membrane Cassette for the appearance of a red line at the "C" control portion and/ or "T" test portion of the window. The red line may appear faint to dark in color. (See Interpretation of Results)

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INTERPRETATION OF RESULTS

- Positive Result: Two red lines are visible, a single red line at the "T" test portion of the Results Window and a single red line at the "C" control portion of the Results Window, indicating the presence of elevated fecal lactoferrin and a properly reactive control.
- Negative Result: A single red line is visible in only the "C" control portion of the Results Window. No red line should be visible at the "T" test portion of the Results Window, indicating the absence of elevated fecal lactoferrin and a properly reactive control.
- Invalid Result: All completed reactions should have a visible red line at the "C" control portion of the Results Window. The test is invalid if a control line is not present or if no lines appear on completed Membrane Cassette.

QUALITY CONTROL

Internal: A red control line must be visible on the "C" side of the Results Window on every Membrane Cassette that is tested. The appearance of the red control line confirms that the sample and reagents were added correctly, that the reagents were active at the time of performing the

assay, and that the sample migrated properly through the Membrane Cassette. A clear background in the result area is considered an internal negative control. If the test had been performed correctly and reagents are working properly, the background will be clear to give a discernible result.

External: The reactivity of the LEUKO EZ VUE® test should be verified on receipt using the Positive Control and negative control (Diluent). The Positive Control is supplied with the kit (red-capped bottle). The Positive Control confirms the reactivity of the other reagents associated with the assay. Diluent is used for the negative control.

- The test should not be used if control tests do not produce the correct results.
- Positive and Negative controls run on each day of patient testing.
- Failure of the internal and external controls to produce the 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 the control tests as the first step in determining the cause of the failure

VISUAL INTERPRETATION OF RESULTS

Positive result

Negative result

Invalid test

Invalid test

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SHELF LIFE AND STORAGE

- The expiration date of the kit is given on the outside of the box.
- Expiration dates for each component are listed on the individual labels.
- The kit containing the reagents should be stored between 2° and 30°C (refrigerated or room temperature).
- Membrane Cassettes should be kept in the sealed pouches until used.

LIMITATIONS OF THE PROCEDURE

1. 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.

2. The 1:50 dilution of fecal specimen 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. There- fore, only the dilution recommended in the brochure should be used.

3. The LEUKO EZ VUE® test has not been clinically evaluated for detecting leukocytes in other types of clinical specimens.

4. The intensity of a positive sample test line does not indicate the amount of lactoferrin or severity of disease.

5. Fecal samples from breast fed infants should not be used with this assay.

Refer to the product insert for performance characteristics

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3. TECHLAB LEUKO EZ VUE. An Immunochromatographic Test for the Qualitative Detection of Elevated Levels of Fecal Lactoferrin. Catalog No. T30355. Issued 07/2016

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Replaces Core Lab/Urinalysis/417/2006/2. Approval and revision of documentation maintained electronically

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