TITLE: LEUKO EZ VUE (Fecal Lactoferrin)

PRINCIPLE/PURPOSE: 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*.

The Leuko EZ VUE test is an immunochromatographic test for the qualitiative detection of elevated levels of fecal lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The Leuko EZ VUE in liquid, semi-solid, and solid fecal specimens. A positive test result indicates an increased level of fecal lactoferrin.

SCOPE: This procedure provides instructions for processing, testing, and result reporting for Alere TechLab LEUKO EZ VUE.

COMPLEXITY LEVEL: Moderate

SAFETY: Gloves lab coat must be worn. Testing is performed in biosafety cabinet.

SPECIMEN:

Patient prep: n/aType: Stool (liquid, formed, or semi-formed)

Acceptable containers: Collect in 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.

Volume required: 50 µL

Stability and storage: Specimens should be stored between 2º and 8ºC or room temperature for up to 2 weeks from time of collection.

Unacceptable Samples: 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.

Handling Conditions: Always us universal procautions and appropriate PPE when handling biololgical specimens.

EQUIPMENT AND MATERIALS: *LEUKO EZ VUE* test kit

Equipment: Pipette 2.5 mL and 300 µL with flared tip, vortex.

Materials: *LEUKO EZ VUE®* test kit: Diluent, cassettes, disposable pipettes, plastic tubes and controls.

Storage Requirements: 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 refrigerated or room temperature. Membrane Cassettes should be kept in the sealed pouches until used.

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 of each new lot number and/or shipment using the Positive Control and Negative Control (Diluent). The Positive Control is supplied with the kit (red-capped bottle). In addition, the external QC is performed 30 days after the kit was opened.

PROCEDURE:

Prepare Diluted Specimen:

Fecal Specimens:

1. 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.
2. 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.
3. Vortex the tubes for 10 seconds before transferring 5 drops of diluted specimen to Sample Well indicated in the diagram of the Membrane Cassette.

Test 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).
6. Document results on log and in LIS.

INTERPRETATION & REPORTING 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 position 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*.

VISUAL INTERPRETATION OF RESULTS

Positive Test Result Negative Test Result

Invalid Test Result Invalid Test Result

Reference Ranges: The prevalence of a positive test result using the *LEUKO EZ VUE*® in clinical investigations ranged between 27% - 53%. The prevalence will vary from location to location and hospitals may experience rates lower or higher than those observed at the sites used in the *LEUKO EZ VUE*® evaluation. The prevalence will vary depending on the incidence of outbreaks due to various enteropathogens

Reporting Format:

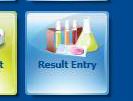
Record results on Patient/QC Log and in LIS.

POS = POSITIVE

NEG = NEGATIVE

ENTERING RESULTS IN LIS:

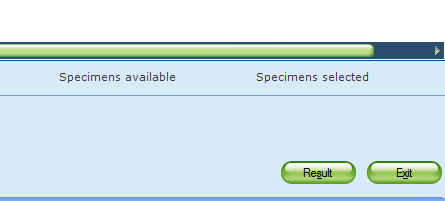
1. Sign into Sunquest.
2. Select “RESULT ENTRY”



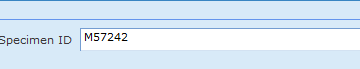
1. Select “MANUAL”
2. Type worksheet “ARHEM”



1. “TAB”
2. “RESULT”



1. Enter accession number.



1. “TAB”
2. Enter lactoferrin result (POS OR NEG)
3. Click “SAVE”
4. Review results.
5. Click “ACCEPT”

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 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.

3. At this time, 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.

REFERENCES:

1. Alere TechLab LEUKO EZ Vue [package insert]. Orlando, FL: TechLab; 2016.

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SOP HISTORY PAGE

SOP Number: MICRO-704-CH

SOP Title: Leuko EZ VUE (Fecal Lactoferrin)

Written By: Jacee Farmer

Manual in which Hard Copy of this SOP is located: Microbiology Procedure Manual

Distribution: SharePoint

Supersedes Procedure: MICRO-708-CH

SOP CHANGE CONTROL

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