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| Fecal Lactoferrin |
| **Purpose** | This procedure provides instruction for the Fecal Lactoferrin test. LEUKO EZ VUE 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. This test detects lactoferrin in liquid, semi-solid and solid fecal specimens. A positive test indicates an increased level of fecal lactoferrin and warrants additional testing. |
| **Principal** | 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 and the other represents a control stripe containing anti-IgG antibodies. The diluted sample and gold conjugate migrate by capillary action when the sample is added to the well. |
| **Policy Statements** | This procedure applies to Microbiologists who work in the Microbiology.  |
| **Test Code** | FLA |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** |  |
|  | LEUKO EZ VUE Kit-Diluent, 65 mL-Membrane Cassettes, 25 per kit-Positive Control, 3.5 mL | LEUKO EZ VUE kit-disposable pipettes-disposable sample preparation devices | * Timer
* Vortex
* 5 ml pipette
* PipetteAid
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| **Specimen** | Fecal specimen in a clean, airtight container with no preservatives. Specimens can be stored at room temperature or refrigerated at 20-80 for 2 weeks.  |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual*and the *Virology Procedure Manual***:**1. *Biohazard Containment*
2. *Safety in the Microbiology/Virology Laboratory*
* *Biohazardous Spills*
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| **Storage and stability** | Store kit and reagents at room temperature or refrigerated (20-300C). Membrane pouches should be kept in the sealed pouches until used. The expiration date for the kit is given on the outside of the box. Expiration dates for each component are listed in the individual labels.  |
| **External****Quality Control** | 1. Perform External Quality Control, Positive and Negative with each new lot, new shipment. The positive control confirms the reactivity of the other reagents in the assay.
2. **Positive Control** is supplied with the kit (red-capped bottle). Add 3 drops into the sample well of the cassette. Read results promptly at 10 minutes.
3. Diluent is used for the **Negative control**. Add 150 µL of Diluent using the transfer pipette into the sample well of the cassette. Read results promptly at 10 minutes.
4. Record in QC binder.
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| **Internal Quality Control** | 1. Record Internal Controls on worksheet FL.
2. **Positive Internal Control**: red control line must be visible in 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, and that the sample migrated properly through the membrane cassette.
3. **Negative Internal Control**: a clear background in the results area is considered an internal negative control. It confirms the test has been performed correctly and the reagents are working properly.
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| **Procedure** | 1. Wearing gloves, vortex specimen thoroughly prior to performing the assay.
2. Set up a single plastic tube for each specimen to be tested.
3. Add 2.5 mL of Diluent to a dilution tube.
4. For liquid fecal specimen, use a transfer pipette to add 50 µL (flared section) and transfer to the dilution tube. This represents a 1:50 dilution of the specimen.
5. For formed/solid fecal specimens, use a transfer pipette to add 0.05 g (flared section) and transfer to the dilution tube. This represents a 1:50 dilution of the specimen.

1. Place filter tip onto the dilution tube with the diluted sample and insert firmly.
2. Vortex the tube for 10 seconds.
3. Open membrane cassette from foil bag.
4. Place membrane cassette on damp paper towels to reduce the effects of static electricity.
5. Label membrane cassette with patient label.
6. Invert and hold the dilution tube vertically, dispense 5 drops into the sample well of the membrane cassette.
7. Incubate the cassette for 10 minutes at room temperature,
8. Read results promptly at 10 minutes. Observe the results window for the appearance of a red line at the “C” control portion and presence or absence of a red line at the “T” test portion
9. The red line may appear faint to dark in color.
10. Specimen and cassettes should be handled with appropriate precautions and dispose of materials in biohazard waste receptacles.
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| **Interpretation/ Results/Critical Values** | 1. **Positive**: two red lines are visible, a single red line at the “T” test potion and single red line at the “C” control portion of the results window indicates the presence of elevated fecal lactoferrin.
2. **Negative**: a single red line is visible in only the “C” control portion of the results window, indicating the absence of elevated fecal lactoferrin.
3. **Invalid**: no red line at the “C” control portion of the results window

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| **Limitations** | 1. Specimens that are in transport media or preserved in formalin or PVA cannot be used.
2. The LEUKO EZ VUE may not be appropriate in immunocompromised persons.
3. The use of lower dilutions may result in positive reactions due to the presence of normal lactoferrin levels. Only the recommend dilution should be used.
4. The intensity of a positive sample test line does not indicate the amount of lactoferrin or the severity of disease.
5. Fecal samples from breast fed infants should not be used.
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| **Method Performance Specifications** | 1. For *in vitro* diagnostic use only.
2. Reagents from the kit box should be at room temperature before use.
3. The pouch containing the membrane cassettes should be opened just before use.
4. Avoid contact with the membrane located in the results window,
5. Reagents from different kits should not be mixed. Do not use the kit beyond the expiration date,
6. Use the dilution of fecal specimen as recommended in the kit.
7. Do no freeze the reagents.
8. 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.
9. Follow all ordinances accordingly for waste disposal regulations.
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| **Result Reporting** | 1. Record results in Sunquest MRE in the Culture Entry tab.
2. Positive test: FLP Positive for Fecal Lactoferrin
3. Negative test: FLN Negative for Fecal Lactoferrin
4. Record patient and QC results on FL worksheet.
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| **References** | TechLab™ Leuko EZ Vue Product Insert. TechLab™ Blacksburg, VA 24060-6358 07/2016Guerrant, R. L. 1992 Measurement of fecal lactoferrin as a marker of fecal leukocytes. J. ClinMicrobiol. 30:1238-1242 |
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | * 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Susan DeMeyere | 7/30/2018 | Initial Version |
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