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| **Applicable Standards** | | | |  | **Version History** | | | |
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| **Related Documents** | | | |  |  |  | |  |
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| **Review History (Up to the Last 15 Occurrences)** | | | | | | | | |
| **Date** | **Version** | | **Revision Type** | | | | **Review By** | |
| 02/01/17 | 1 | | New Policy/Procedure | | | |  | |
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| **TITLE:** | **LEUKO EZ VUE** | | | |
| **Effective Date:** | | 02/01/2017 | **Department:** | Microbiology |
| **Dates(s) Revised:** | |  | **SOP number:** | BM0325 |
|  | |  | **Revised By:** | Jessica Mercer MLS (ASCP)CM |
|  | |  |  |  |
| **Approved By:** | | Randall L. Simonsen, MD | | Medical Director |
| (Original signed copy available in the laboratory Microbiology Policy and Procedure Manual) | | | | |

**INTENDED USE**

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*.*

FOR *IN VITRO* DIAGNOSTIC USE.

**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*.

**REAGENTS**

***Diluent***, 65 mL (Ready-to-use, contains phosphate-buffered saline, detergent and 0.1% sodium azide)

***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****,* 2.0 mL (phosphate-buffered saline containing purified human lactoferrin and 0.1% sodium azide)

***Disposable plastic pipettes***, 25 (flared section = 50 μL)

***Disposable sample preparation devices***, 25 (25 tubes and 25 filter tips)

**PRECAUTIONS**

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

2. Pouch containing *Membrane Cassette* should be opened just before use.

3. Keep the *Membrane Cassettes* dry before use.

4. Specimens and *Membrane Cassettes* should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.

5. Reagents contain sodium azide as a preservative and should be handled with normal laboratory caution.

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

7. 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.

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

9. All *Membrane Cassettes* must be read promptly at 10 minutes.

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

**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 *Results Window*.

**COLLECTION AND HANDLING OF FECAL SPECIMENS**

**NOTE**: 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.**

1. 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.

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

**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)

**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, and is not intended to ensure precision at the analytical assay cut-off. *Diluent* is used for the negative control.

Additional tests including External Controls should be performed to meet the requirements of local, state and/or federal regulations and/or accrediting organizations. The reactions expected with the external controls are described in the section on INTERPRETATION OF RESULTS. The test should not be used if control tests do not produce the correct results. Proper results obtained with the internal control line, the *Positive Control* and negative control (*Diluent*) serve as indicators that the test was performed correctly,

that the antibodies striped on the membrane and the *Conjugate* are active at the time of testing, and that the cassette supports proper sample fl ow. 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.

**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.

**EXPECTED VALUES**

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.

**LIMITATIONS OF THE PROCEDURE**

1. The *LEUKO EZ VUE®* test detects elevated levels of lactoferrin released from fecal leukocytes as a marker of intestinal infl ammation. 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.**

**CROSS-REACTIVITY**

Various intestinal organisms were examined for cross-reactivity in the *LEUKO EZ VUE®* test. For the analysis, broth cultures mixed 1:50 with 1X *Diluent* were evaluated. Broth cultures at log phase containing 108 bacteria per mL were used. No cross-reactivity was observed with any of the microbial organisms listed in Table 2.

Table 2. Microbial organisms tested in the *LEUKO EZ VUE*® test.

*Acinetobacter lwoffi I Aeromonas hydrophila Bacillus cereus*

*Bacillus subtilis Bacteroides distasonis Bacteroides eggerthii*

*Bacteroides fragilis Bacteroides ovatus Bacteroides stercoris*

*Bacteroides thetaiotaomicron Bacteroides uniformis*

*Bacteroides vulgatus Bifidobacteri umadolescentis Bifidobacterium longum Campylobacter jejuni Candida albicans Candida krusei*

*Candida tropicalis Clostridium bifermentans Clostridium chauvoei Clostridium diffi cile*

*Clostridium haemolyticum Clostridium histolyticum Clostridium novyi* (types A,B,C)

*Clostridium perfringens* (types A,B,C,D,E) *Clostridium septicum*

*Clostridium sporogenes Clostridium tetani Enterococcus faecalis*

*Escherichia coli Eubacterium aerofaciens Fusobacterium prausnitzii*

*Klebsiella pneumoniae Peptostreptococcus anaerobius*

*Proteus vulgaris Pseudomonas aeruginosa Salmonella choleraesuis*

*Salmonella enteritidis Salmonella typhi Salmonella typhimurium*

*Shigella dysenteriae Shigella fl exneri Shigella sonnei*

*Staphylococcus aureus Vibrio parahaemolyticus Yersinia enterocolitica*

Adenovirus type 1 (ATCC #VR-1) Adenovirus type 2 (ATCC #VR-846)

Adenovirus type 3 (ATCC #VR-3) Adenovirus type 5 (ATCC #VR-5)

Adenovirus type 40 (ATCC #VR-931) Human coronavirus (ATCC #VR-740)

Enterovirus type 70 (VR-836) Coxsackievirus B2 (VR-29)

Coxsackievirus B2 (VR-30) Coxsackievirus B2 (VR-184)

Coxsackievirus B2 (VR-185) Echovirus 18 (VR-48)

Echovirus 33 (VR-582) Enterovirus type 70 (VR-784)

**EFFECT OF FECAL SAMPLE CONSISTENCY**

The *LEUKO EZ VUE*® test detects lactoferrin in liquid, semi-solid, and solid fecal specimens.

**REPRODUCIBILITY AND PRECISION**

The inter-assay variation was determined by analyzing 7 lactoferrin-negative and 13 lactoferrin-positive fecal specimens over a 3-day period. There was 100% correlation for both the positive specimens and negative specimens including samples close to the cut-off of the kit. The intra-assay variation was determined by analyzing 19 fecal specimens using

6 replicates in a single kit lot. There was a 100% correlation between results for the intraassay analysis.

**INTERFERING SUBSTANCES**

The following substances had no effect on test results when present in feces in theconcentrations indicated: mucin (5% w/v), serum containing lipid (fecal fats, 5% v/v),Mylanta (5% v/v), Pepto-Bismol (5% v/v), Imodium (5% v/v), Kaopectate (5% v/v),Bilirubin (5% w/v), Hemoglobin (10 mg/g feces).

FOR INFORMATIONAL USE

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Revised by: Jessica Mercer MT (ASCP)CM  01/17/17