

# IBD EZ VUE®

An Immunochromatographic Test for the Qualitative Detection  
of Elevated Levels of Fecal Lactoferrin.

Catalog No. T5018 (25 Tests)

Distributed by



NL Charles Stulemeijerweg 15, 5026 RS Tilburg  
T 013 467 27 00 F 013 467 80 16  
B Rond Point Schuman 6, 1040 Brussels  
T 02 280 05 06 F 02 280 06 61  
orange@orangemedical.nl www.orangemedical.nl

Developed and Manufactured by



TECHLAB®




U. S. Patent # 7,192,724  
Australian Patent # 2002220029


International Symbol Key:

**REF** Catalog Number

**IVD** *In Vitro* Diagnostic Medical Device


**LOT** Lot Information

 Contains sufficient reagents  
for <n> tests

 Temperature Limitation

 Use By/Expiration Date

**CE** CE Symbol

 Caution, consult  
accompanying documents

## IBD EZ VUE®

### INTENDED USE

The *IBD EZ VUE*® test is an immunochromatographic test for the qualitative detection of elevated levels of lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The test can be used as an *in vitro* diagnostic aid to help identify patients with active inflammatory bowel disease (IBD) and rule out those with active noninflammatory irritable bowel syndrome (IBS).  
FOR *IN VITRO* DIAGNOSTIC USE.

### EXPLANATION

Inflammatory bowel disease is considered a condition of chronic inflammation. Ulcerative colitis and Crohn's disease both exhibit large numbers of leukocytes that migrate to the mucosa and into the intestinal lumen. Endoscopic examination may be used to identify inflamed intestinal mucosa in patients with IBD (3). During the diagnosis of IBD, efforts must be made to rule out other more common etiologies such as infectious colitis (e.g., those caused by *Shigella*, *Campylobacter*, and *Clostridium difficile*) (2,7). Patients with active IBD but exhibiting mild signs and symptoms may be difficult to distinguish from patients with active IBS. Unlike IBD, IBS does not involve intestinal inflammation. In persons with IBS, the intestine appears normal upon endoscopic examination and leukocytes are not present in the mucosa or in fecal specimens (1).

Human lactoferrin is an 80 kilodalton glycoprotein detected by the *IBD EZ VUE*® test. This iron-binding protein is secreted by most mucosal membranes and is a major component of the secondary granules of leukocytes, a primary component of the acute inflammatory response. Other hematopoietic cells such as monocytes and lymphocytes do not contain lactoferrin whereas various bodily secretions contain levels in the mg/mL range. During intestinal inflammation, leukocytes infiltrate the mucosa, increasing the level of fecal lactoferrin (4-10). The *IBD EZ VUE*® test is a rapid noninvasive test that detects elevated fecal lactoferrin as a marker of intestinal inflammation. Test results may be used as an aid for the differentiation of active IBS from active IBD.

### PRINCIPLE OF THE TEST

The *IBD EZ VUE*® test utilizes 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 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

DIL   SPE	<b>Diluent</b> , 65 mL (Ready-to-use, contains phosphate-buffered saline, detergent and 0.10% sodium azide)
MEM   CAS	<b>Membrane Cassettes</b> , 25 (1 <i>Membrane Cassette</i> per pouch; each membrane is coated with anti-lactoferrin antibodies and contains antibodies conjugated to colloidal gold)
CONTROL   +	<b>Positive Control</b> , 1.0 mL (phosphate-buffered saline containing purified human lactoferrin and 0.10% sodium azide)

**Transfer 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. The pouch containing the *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 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

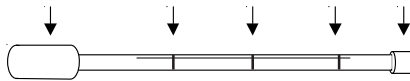
**NOTE:** Collect fecal specimens into a clean, airtight container with no preservatives. Specimens should be stored between 2° and 8°C or room temperature for up to 2 weeks from time of collection then stored frozen at -20°C or lower. Diluted specimens should be stored between 2° and 8°C or at room 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.**

### 1. Prepare Diluted Specimen.

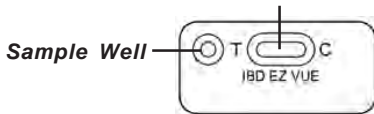
**Fecal Specimens:** Set up a single plastic tube for each specimen to be tested. For each specimen, add 2.5 mL of *Diluent* to a dilution tube. Use a transfer pipette to add 50 µL (flared section) of liquid fecal specimen or weigh 0.05g of a solid fecal specimen to add to the tube. Next, place a filter 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 4 drops of diluted specimen to sample well indicated in the diagram of the *Membrane Cassette*.

**Transfer Pipette:** Bulb      300  $\mu$ L    200  $\mu$ L    100  $\mu$ L    50  $\mu$ L Flared tip



**Membrane Cassette Diagram**  
**Results Window**



**PROCEDURE**

1. Remove required number of *Membrane Cassettes*, one per specimen, from the foil bags containing desiccant.
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 **4 drops** (150  $\mu$ L) into the *Sample Well* of a *Membrane Cassette*. If running an external control, each control requires the addition of **3 drops** (150  $\mu$ L) from the dropper bottle or pipette (held vertically) into the sample well of the cassette.  
(NOTE: *Diluent* is used as the negative external control)
4. Incubate each *Membrane Cassette* for 10 minutes at room temperature.
5. Read results: 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.

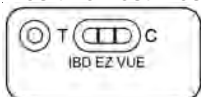
**External:** The reactivity of the *IBD EZ VUE*<sup>®</sup> test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (red-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated 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 reactive at the time of testing, and that the device supports proper sample flow. Failure of the internal and/or 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 Test Result



#### Negative Test Result



#### Invalid Test Result



#### Invalid Test Result



### 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.

### PERFORMANCE CHARACTERISTICS

There were 23 ulcerative colitis patients, 70 Crohn's disease patients, 17 irritable bowel patients, and 27 healthy persons recruited from two different IBD referral centers and TECHLAB®, Inc. All 12 patients with active ulcerative colitis (100%) were positive in the *IBD EZ VUE*® test. There were 11 patients with inactive ulcerative colitis and none of these were positive in the *IBD EZ VUE*® test. All 46 patients with active Crohn's disease (100%) were positive in the *IBD EZ VUE*® test. There were 24 patients with inactive Crohn's disease and of these 2 (8.3%) were positive. All 17 irritable bowel patients (100%) and all 27 healthy persons (100%) were negative in the *IBD EZ VUE*® test. The values when comparing the *IBD EZ VUE*® test to the *IBD-CHEK*® test and for distinguishing active ulcerative colitis (UC) and active Crohn's disease (CD) from active irritable bowel syndrome (IBS) and healthy persons are shown in the following table.

#### Statistical analysis of the *IBD EZ VUE*®

Value	<i>IBD-CHEK</i> ® vs <i>IBD EZ VUE</i> ®	Active UC vs IBS and healthy persons	Active CD vs IBS and healthy persons
Sensitivity	100%	100%	100%
Specificity	97.5%	100%	100%
Predictive Positive Value	96.7%	100%	100%
Predictive Negative Value	100%	100%	100%
Correlation	98.5%	100%	100%

### LIMITATIONS OF THE PROCEDURE

1. The *IBD EZ VUE*<sup>®</sup> test is a screening test that 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 *IBD EZ VUE*<sup>®</sup> 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.

### CROSS-REACTIVITY

Various intestinal organisms were examined for cross-reactivity in the *IBD EZ VUE*<sup>®</sup> test. For the analysis, broth cultures mixed 1:50 with 1X *Diluent* were evaluated. Broth cultures at log phase containing  $\geq 10^8$  bacteria per mL were used. No cross-reactivity was observed with any of the organisms tested.

### EFFECT OF FECAL SAMPLE CONSISTENCY

The *IBD EZ VUE*<sup>®</sup> test detected lactoferrin in liquid, semi-solid, and solid fecal specimens at levels similar to those observed with purified lactoferrin prepared in kit *Diluent*.

### REPRODUCIBILITY AND PRECISION

The inter-assay variation was determined by analyzing 9 lactoferrin-negative and 10 lactoferrin-positive fecal specimens over a 3-day period. There was 100% correlation for both the positive specimens and negative specimens. 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 intra-assay analysis. A total of 3 Physician's offices evaluated the *IBD EZ VUE*<sup>®</sup> test for reproducibility using 10 lactoferrin-positive and 10 lactoferrin negative fecal specimens. Overall correlations for test sites as compared to results generated at TECHLAB<sup>®</sup>, Inc. ranged from 90 to 100%.