LACTOFERRIN CHEK®

An ELISA for the qualitative detection of
elevated levels of fecal lactoferrin
Catalog No. 30352 (96 Tests)
IVD *In Vitro* Diagnostic Medical Device

Made in the USA

U. S. Patent # 7,192,724

Developed and Manufactured by:

TECHLAB®

2001 Kraft Drive
Blacksburg, VA 24060-6358 USA
www.techlab.com
+1 800 TECHLAB
(540) 953-1664

LACTOFERRIN CHEK®

INTENDED USE

The LACTOFERRIN CHEK® test is an ELISA 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 irritable bowel syndrome (IBS), which is noninflammatory.

Caution: U.S. Federal Law restricts this device to sale by or on the order of a physician.

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 utilized by the LACTOFERRIN CHEK® test. This iron-binding protein is secreted by most mucosal membranes and is a major component of the secondary granules of polymorphonuclear neutrophils (PMN), 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).

Clinical studies support the use of the LACTOFERRIN CHEK® test as an *in vitro* diagnostic aid for detecting elevated levels of lactoferrin, a marker of fecal leukocytes and an indicator of intestinal inflammation. Further, our results support the use of the LACTOFERRIN CHEK® test as an *in vitro* diagnostic aid to help distinguish active IBD patients from those with active IBS.

PRINCIPLE OF THE TEST

The LACTOFERRIN CHEK® test uses antibodies to human lactoferrin. The microassay wells supplied with the kit contain immobilized polyclonal antibody against lactoferrin. The detecting antibody consists of polyclonal antibody conjugated to horseradish peroxidase. In the assay, an aliquot of fecal specimen is emulsified in the *Diluent* and the diluted specimen is transferred to the microassay well. If elevated levels of lactoferrin are present in the specimen, the lactoferrin binds to the immobilized antibody. After incubation, the wells are washed and the antibody conjugate is added. The conjugate binds to the lactoferrin bound during the first incubation phase. A second series of wash steps removes any unbound material. Following the addition of *Substrate*, a color is detected due to the enzyme-antibody-antigen complexes that form in the presence of lactoferrin.

REAGENTS

DIL | 10X — ***10X Diluent***, 40 mL (10X concentrate of a buffered protein solution containing 0.2% thimerosal). The 1X *Diluent* is also to be used as the negative control (see TEST PROCEDURE).*

Signal Word: Warning

H373: May cause damage to organs through prolonged or repeated exposure

H411: Toxic to aquatic life with long lasting effects

P260, P273, P314, P391, P501

CONJ | ENZ — ***Conjugate***, 7 mL (rabbit polyclonal antibody specific for human lactoferrin

conjugated to horseradish peroxidase and in a buffered protein solution containing 0.02% thimerosal)*

SUBS | REAG ***Substrate***, 14 mL (solution containing tetramethylbenzidine substrate and peroxide)

CONTROL | + ***Positive Control***, 3.5 mL (human lactoferrin in a buffered protein solution containing 0.02% thimerosal)*

WASHBUF | 20X ***Wash Buffer Concentrate***, 50 mL (20X concentrate containing phosphate-buffered saline, detergent and 0.2% thimerosal)*

Signal Word: Warning

H373: May cause damage to organs through prolonged or repeated exposure

H411: Toxic to aquatic life with long lasting effects

P260, P273, P314, P391, P501

H_2SO_4 | 0.6N ***Stop Solution***, 7 mL (0.6 N sulfuric acid). CAUTION: Avoid contact with skin. Flush with water immediately if contact occurs.

Signal Word: Danger

H314: Causes severe skin burns and eye damage

P260, P264, P280, P301, P330, P331, P303, P361, P353, P363, P304, P340, P310, P321, P305, P351, P338, P501

MA | PLT ***Microassay Plate***, 12 strips, 8 wells per strip, coated with purified polyclonal antibody specific for lactoferrin (stored with desiccant)

*contains mercury (Hg)

PRECAUTIONS

1. Rx Only - Prescription Only
2. For *in vitro* diagnostic use. For professional use only.
3. Reagents from different kits should not be mixed. Do not use the kit past the expiration date.
4. Reagents should be at room temperature before use.
5. Gently mix all reagents before use.
6. Caps and tips are color-coded; do not mix!
7. When handling the microassay wells, avoid scratching the bottom of the wells because this may result in elevated absorbance readings.
8. Hold dropper bottles vertically to ensure proper drop size.
9. Handle specimens and used microassay wells as if capable of transmitting infectious agents. Wear gloves when doing the test.
10. *Unused microassay wells must be placed inside the resealable pouch with the desiccant to protect them from moisture.*
11. Perform the washing procedure as directed to avoid high background reactions.
12. Frozen specimens (-20°C or lower) may lose activity due to freezing and thawing multiple times.
13. Do not freeze the reagents. Store the kit between 2°C and 8°C.
14. The *Substrate* is light sensitive and should be protected from direct sunlight or UV sources.
15. Optimal results are obtained by following the specified test procedure. The concentrations, incubation conditions, and processing specifications have been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. 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.
16. 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*.

17. The *10X Diluent* and the *20X Wash Buffer Concentrate* contain 0.2% thimerosal as a preservative. Once diluted to normal use concentration these solutions are classified as non-hazardous. The *Stop Solution* contains 0.6 N sulfuric acid. Flush with water immediately if contact occurs. 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.
18. Follow your national, regional, and local ordinances accordingly for waste disposal regulations. Do not place in trash, dispose of as hazardous waste.

PRELIMINARY PREPARATIONS

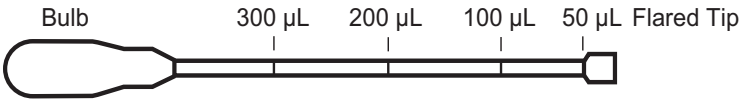
1. All reagents must be at room temperature prior to use in the assay.
2. **Prepare 1X *Wash Solution.*** The *Wash Solution* is supplied as a 20X concentrate (a precipitate may be noticed). Dilute to a total volume of 1 liter by adding 50 mL of the concentrate to 950 mL of deionized water. Label the bottle. Store any unused 1X *Wash Solution* between 2°C and 8°C.
3. **Prepare 1X *Diluent*.** The *Diluent* is supplied as a 10X concentrate. Dilute to a total volume of 400 mL by adding 40 mL of the concentrate to 360 mL of deionized water. Label the bottle. Store any unused 1X *Diluent* between 2°C and 8°C.
4. ***Microassay Plate preparation*.** Each Strip contains 8 wells coated with polyclonal antibody specific for lactoferrin. Each specimen or control will require one of these coated wells. Avoid contact with the bottom of the wells because this is the optical window for ELISA readers. Microassay wells not used must be returned to the plastic bag and carefully resealed with desiccant.

COLLECTION OF SPECIMENS AND PREPARATION OF DILUTIONS

NOTE: Collect fecal specimens into a clean, airtight container. Do not use specimens that have been collected or stored in 10% formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol fixatives. Specimens should be stored between 2° and 8°C or room temperature for up to 2 weeks from collection then stored frozen at -20°C or lower. Diluted specimens should be stored between 2° and 8°C 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.**
   For Liquid Fecal Specimens: Set up two plastic tubes for each specimen to be tested. For each specimen, add 950 µL of 1X *Diluent* to each of the two tubes. Use a transfer pipette to add 50 µL (flared section) of liquid fecal specimen to one of the tubes and mix well using a vortex mixer. Next, transfer 50 µL (flared section) of the previously diluted specimen into the second tube containing 950 µL of 1X *Diluent*. This represents a 1:400 dilution of the specimen and only the second tube should be used for the remainder of the test.
   For Formed/Solid Fecal Specimens: Set up two plastic tubes for each specimen to be tested. For each specimen, add 950 µL of 1X *Diluent* to each of the two tubes. Use a transfer pipette to add 0.05 g (flared section) or weigh 0.05g of fecal specimen and add to the tube containing *Diluent* (1:20). Mix well using a vortex mixer. Next, transfer 50 µL (flared section) of the previously diluted specimen into the second tube. This represents a 1:400 dilution of the specimen and only the second tube should be used for the remainder of the test.
2. Vortex the tubes for 10 seconds and store between 2° and 8°C until the test is performed. Vortex again before transferring diluted specimen to microassay well. This ensures thorough mixing of the specimen.

Transfer Pipette:



TEST PROCEDURE

Materials provided

2 Plastic adhesive sheets
100 transfer pipettes (flared section = 50 µL)

Materials and equipment required but not provided

Squirt bottle for *1X Wash Solution*
Vortex mixer
Refrigerator for storage
Tubes for dilution of specimen
Incubator set at 37°C ± 2°C
Discard container/absorbent paper
Bottle for diluted *1X Diluent*
Deionized or distilled water
ELISA reader capable of reading 450 nm or 450/620 nm

1. Determine the number of wells to be used. Add 1 drop of *Positive Control* (black cap) to a positive control well. Use a transfer pipette to add 50 µL (flared section) of 1X *Diluent* to a negative control well. Use a transfer pipette to add 100 µL (first mark past flared section) of 1:400 diluted specimen to one well.
2. Incubate the wells at 37°C ± 2°C for 30 minutes.
3. Shake out the contents of the assay wells into a discard pan.
4. Wash each well using the diluted *Wash Solution* in a squirt bottle with a fine-tipped nozzle, directing the 1X *Wash Solution* to the bottom of the well with force. Fill the wells, then shake the *Wash Solution* out of the well into a discard pan. Slap the inverted plate on a dry paper towel and repeat wash steps **four times** using a dry paper towel each time. If any particulate matter is seen in the wells, continue washing until all the matter is removed.
5. Add 1 drop of *Conjugate* (red cap) to each well. Incubate the wells at 37°C ± 2°C for 30 minutes.
6. Repeat step #3 and #4. Dispose of all paper towels and specimen containers properly.
7. Add 2 drops of *Substrate* (blue cap) to each well. Gently tap the wells to mix the contents. Incubate the wells at room temperature for 15 minutes. Gently tap the wells 1 or 2 times during the incubation period.
8. Add 1 drop of *Stop Solution* (yellow cap) to each well. Gently tap the wells and wait 2 minutes before reading. The addition of *Stop Solution* converts the blue color to a yellow color which may be quantitated by measuring the optical density at 450 nm on a microplate ELISA reader. Wipe the underside of each well before measuring the optical density. If a dual reader is used, read at 450 nm and reference 620 nm. Read within two to ten minutes after adding *Stop Solution.*
9. Record absorbance values for the positive control, negative control, and each specimen tested.
10. Average the readings of duplicate wells before interpreting results.

QUALITY CONTROL

The positive and negative control must meet the following criteria or the test is not valid. The positive control well must be a visible yellow color. When read on a spectrophotometer, it must have an OD_{450} and $OD_{450/620}$ nm >0.500. The negative control must have an OD_{450} <0.200 or an $OD_{450/620}$ nm <0.160. To ensure that carryover did not occur, repeat testing if a sample gives a weak positive result (i.e., <0.400) and is adjacent to a strong positive well.

INTERPRETATION OF RESULTS

Measurements should be determined at 450 nm on a single wavelength spectrophotometer and at 450/620 nm on a dual wavelength spectrophotometer.

1. **Spectrophotometric Single Wavelength at 450 nm**
   Negative = OD_{450} <0.200
   Positive = OD_{450} ≥0.200
2. **Spectrophotometric Dual Wavelength at 450/620 nm**
   Negative = $OD_{450/620}$ <0.160
   Positive = $OD_{450/620}$ ≥0.160

A positive test result indicates the specimen contains elevated levels of lactoferrin. A negative test result indicates the specimen does not contain elevated levels of lactoferrin.

SHELF-LIFE AND STORAGE

The expiration date of the kit is given on the outside label. Expiration dates for each component are listed on the individual labels. The kit containing the reagents with designated shelf-life should be stored between 2° and 8°C and should be returned to the refrigerator as soon as possible after use.

PERFORMANCE CHARACTERISTICS

There were 71 ulcerative colitis patients, 78 Crohn's disease patients, 31 irritable bowel patients, and 56 healthy persons recruited from three different IBD referral centers (one on the east coast and two in the Midwest) and TECHLAB®, Inc. Of the 40 patients with active ulcerative colitis, 35 (88%) were positive in the LACTOFERRIN CHEK® test. There were 31 patients with inactive ulcerative colitis and 16 (52%) of these were positive in the LACTOFERRIN CHEK® test. Of the 52 patients with active Crohn's disease, 44 (85%) were positive in the LACTOFERRIN CHEK® test. There were 26 patients with inactive Crohn's disease and of these 16 (62%) were positive. All of the 31 irritable bowel patients (100%) and all 56 healthy persons (100%) were negative in the LACTOFERRIN CHEK® test. The values when distinguishing active IBD, 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.

Value	Active IBD vs IBS and healthy persons	Active UC vs IBS and healthy persons	Active CD vs IBS and healthy persons
Sensitivity	86%	88%	85%
Specificity	100%	100%	100%
Predictive Positive Value	100%	100%	100%
Predictive Negative Value	87%	95%	92%
Correlation	93%	96%	94%

LIMITATIONS OF THE PROCEDURE

1. The LACTOFERRIN CHEK® test is a screening test that detects elevated levels of lactoferrin released from fecal leukocytes. The test may not be appropriate in immunocompromised persons. The following patient samples should be excluded from use in the LACTOFERRIN CHEK® test: patients with a history of HIV and/or Hepatitis B and C, patients with a history of infectious diarrhea (within 6 months), and patients having had a colostomy and/or ileostomy within 1 month.
2. The dilutions of fecal specimen recommended in the brochure have been evaluated in clinical trials and have been found to be optimal for test results. The use of lower dilu-

tions may result in positive reactions due to the presence of normal lactoferrin levels. Therefore, only the dilutions recommended in the brochure should be used.

3. At this time, the LACTOFERRIN CHEK® test has not been clinically evaluated for use in the detection of leukocytes in other types of clinical specimens. Use the test only for the analysis of fecal specimens.
4. Fecal specimens that have been preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin, or polyvinyl alcohol cannot be used.
5. Patients with IBD oscillate between active (inflammatory) and inactive (noninflammatory) stages of disease. These stages must be considered when using the LACTOFERRIN CHEK® test.
6. Other intestinal ailments, including many gastrointestinal infections and colorectal cancer, often result in elevated levels of lactoferrin in fecal specimens and these specimens will test positive with the LACTOFERRIN CHEK® test. Therefore, a diagnosis of active IBD cannot be established solely on the basis of a positive result with the LACTOFERRIN CHEK® test.

CROSS-REACTIVITY

Various intestinal organisms were examined for cross-reactivity in the LACTOFERRIN CHEK® test. For the analysis, broth cultures mixed with *1X Diluent* were evaluated. Broth cultures at log phase containing >10^8 bacteria per mL were used. Organisms that did not react in the LACTOFERRIN CHEK® test are as follows:

Acinetobacter lwoffii
Aeromonas hydrophila
Bacillus cereus
Bacillus subtilis
Bacteroides distasonis
Bacteroides eggerthii
Bacteroides fragilis
Bacteroides ovatus
Bacteroides stercoris
Bacteroides thetaiotaomicron
Bacteroides uniformis
Bacteroides vulgatus
Bifidobacterium adolescentis
Bifidobacterium longum
Campylobacter jejuni
Candida albicans
Candida krusei
Candida tropicalis
Clostridium bifermentans
Clostridium chauvoei
Clostridium difficile
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
Eubacterium aerofaciens
Escherichia coli
Fusobacterium prausnitzii
Klebsiella pneumoniae
Peptostreptococcus anaerobius
Proteus vulgaris
Pseudomonas aeruginosa
Salmonella choleraesuis
Salmonella enteritidis
Salmonella typhi
Salmonella typhimurium
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Vibrio parahaemolyticus
Yersinia enterocolitica

EFFECT OF FECAL SAMPLE CONSISTENCY

The LACTOFERRIN CHEK® test detected lactoferrin in liquid, semi-solid, and solid fecal specimens at similar levels to those observed with purified lactoferrin prepared in kit *Diluent*.

REPRODUCIBILITY AND PRECISION

The inter-assay variation was determined by analyzing 8 lactoferrin-negative and 8 lactoferrin-positive fecal specimens over a 3 day period. The average %CV was 23.5% for the positive specimens and 7.4% for the negative specimens. The intra-assay variation was determined by analyzing 12 fecal specimens using 6 replicates in one lot of kits. The intra-assay analysis ranged in %CV from 2.7 to 24.0 with an average of 8.7%.

REFERENCES CITED

1. Camilleri, M. 2001. Management of the irritable bowel syndrome. Gastroenterology 120:652-668.
2. Everhart, J. E. 1994. Digestive diseases in the United States: epidemiology and impact. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. U.S. Government Printing Office, NIH Publication no. 94-1447.
3. Fine, K. D., F. Ogunji, J. George, M. Niehaus, and R. Guerrant. 1998. Utility of a rapid fecal latex agglutination test detecting the neutrophil protein lactoferrin, for diagnosing inflammatory causes of chronic diarrhea. Am. J. Gastroenterol. 93:1300-1305.
4. Guerrant, R.L., V. Araujo, E. Soares, K. Kotloff, A. Lima, W. Cooper, and A. Lee. 1992. Measurement of fecal lactoferrin as a marker of fecal leukocytes. J. Clin. Microbiol. 30:1238-1242.
5. Harris, J. C., H. L. DuPont, and B. R. Hornick. 1971. Fecal leukocytes in diarrheal illness. Ann. Intern. Med. 76:697-703.
6. Riley, L. W. 1995. Acute inflammatory diarrhea. In M. Blaser, P. Smith, J. Ravdin, H. Greenberg, and R. Guerrant (ed.), *Infections of the Gastrointestinal Tract*. Raven Press, New York, NY.
7. Sartor, R. B. 1995. Microbial agents in pathogenesis, differential diagnosis, and complications of inflammatory bowel disease. In M. Blaser, P. Smith, J. Ravdin, H. Greenberg, and R. Guerrant (ed.), *Infections of the Gastrointestinal Tract*. Raven Press, New York, NY.
8. Sugi, K., O. Saitoh, I. Hirata, and K. Katsu. 1996. Fecal lactoferrin as a marker for disease activity in inflammatory bowel: comparison with other neutrophil derived proteins. Am. J. Gastroenterol. 91:927-934.
9. Uchida, K., R. Matsuse, S. Tomita, K. Sugi, O. Saitoh, and S. Ohshiba. 1994. Immunochemical detection of human lactoferrin in feces as a new marker for inflammatory gastrointestinal disorders and colon cancer. Clin. Biochem. 27:259-264.
10. Washington, J. A., and G. V. Doern. 1991. Assessment of new technology. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of Clinical Microbiology*. American Society for Microbiology.

Technical Support

Further information can be obtained by contacting TECHLAB® Technical Support:

US	+1 800 TECHLAB
Phone	(540) 953-1664
Fax	(540) 953-1665
Email	ts@techlab.com

RMS #91-352-02-TL **Issued: 07/2021**