This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21 CFR 809.10).

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the package insert. Any modifications to this document are the sole responsibility of the Laboratory.

QuickVue TLI Lactoferrin

CLIA Complexity: Moderate

For *in vitro* diagnostic use only. 

For Canadian Users: For Laboratory Use Only

A symbols glossary can be found at quidel.com/glossary.

INTENDED USE

The QuickVue TLI Lactoferrin 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). **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 detected by the QuickVue TLI Lactoferrin 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 QuickVue TLI Lactoferrin 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 QuickVue TLI Lactoferrin 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

Membrane Cassettes – 25, 1 *Membrane Cassette* per pouch Each membrane is coated with anti-lactoferrin antibodies and contains antibodies conjugated to colloidal gold	MEM CAS
Diluent (65 mL) – Ready-to-use, contains phosphate-buffered saline, detergent and 0.05% ProClin® 300)* Signal Word: Warning H317: May cause an allergic skin reaction P261, P272, P280, P302, P352, P333, P313, P321, P362, P364, P501 	DIL SPE
Positive Control (3.5 mL) – Phosphate-buffered saline containing purified human lactoferrin	CONTROL +
Transfer pipettes (25) – Flared section = 50 µL	
Disposable sample preparation devices (25) – 25 tubes and 25 filter tips	

PRECAUTIONS

1. Rx Only – Prescription Only
2. Reagents from the kit box should be at room temperature before use.
3. The pouch containing the *Membrane Cassette* should be opened just before use.
4. Keep the *Membrane Cassettes* dry before use.
5. Reagents from different kits should not be mixed. Do not use the kit past the expiration date.
6. 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.
7. Do not freeze the reagents. The kit should be stored between 2°C and 30°C.
8. All *Membrane Cassettes* must be read promptly at 10 minutes.
9. To minimize the effects of static electricity, place all *Membrane Cassettes* with *Results Window* facing upwards on damp paper towels.
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. Specimens and *Membrane Cassettes* should be handled and disposed of as potential biohazards after use.
13. Wear disposable gloves when doing the test.
14. 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. Handle reagents according to existing regulations for laboratory safety and good laboratory practice.
15. Follow your national, regional, and local ordinances accordingly for disposal regulations.
16. For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) available from Technical Support at technicalsupport@quidel.com.

PRELIMINARY PREPARATIONS

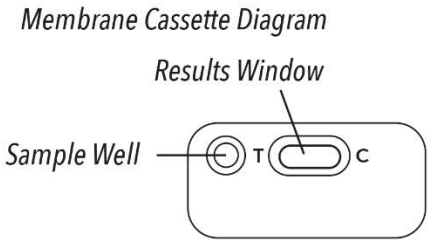
1. All reagents must be removed from the kit box and allowed to reach room temperature prior to use in the assay.
2. ***Membrane Cassette preparation.*** Each pouch contains 1 *Membrane Cassette* coated with polyclonal antibody specific for lactoferrin. Each specimen or control will require one of these *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°C 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°C 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. 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°C 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.*
   Transfer Pipette:



PROCEDURE

1. 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 the *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 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

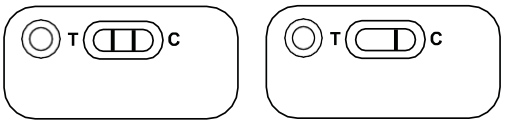
The reactivity of the QuickVue TLI Lactoferrin Test should be verified on receipt using the *Positive Control* and negative control (*Diluent*). The *Positive Control* is supplied with the kit (red-capped bottle). The *Positive Control* confirms the reactivity of the other reagents associated with the 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.

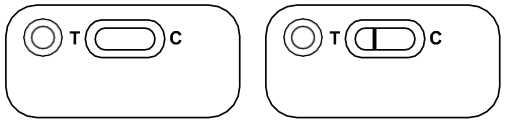
The reactions expected with the external controls are described in the section on INTERPRETATION OF RESULTS. The test should not be used if control tests do not produce the correct results. Proper results obtained with the internal control line, the *Positive Control* and negative control (*Diluent*) 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°C 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 QuickVue TLI Lactoferrin Test. There were 11 patients with inactive ulcerative colitis and none of these were positive in the QuickVue TLI Lactoferrin Test. All 46 patients with active Crohn's disease (100%) were positive in the QuickVue TLI Lactoferrin 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 QuickVue TLI Lactoferrin Test. The values when comparing the QuickVue TLI Lactoferrin Test to the LACTOFERRIN 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 QuickVue TLI Lactoferrin Test

Value	QuickVue TLI Lactoferrin vs LACTOFERRIN CHEK®	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

- The QuickVue TLI Lactoferrin 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.
- 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.
- At this time, the QuickVue TLI Lactoferrin Test has not been clinically evaluated for detecting leukocytes in other types of clinical specimens.

- 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 QuickVue TLI Lactoferrin Test. For the analysis, broth cultures mixed 1:50 with 1X *Diluent* were evaluated. Broth cultures at log phase containing ≥10^8 bacteria per mL were used. No cross-reactivity was observed with any of the organisms tested.

EFFECT OF FECAL SAMPLE CONSISTENCY

The QuickVue TLI Lactoferrin 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 QuickVue TLI Lactoferrin 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, Inc. ranged from 90 to 100%.

REFERENCES

1. Camilleri, M. 2001. Management of the irritable bowel syndrome. Gastroenterol.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 andKidney 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. Gastroenterology. 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. Kayazawa, M., O. Saitoh, K. Kojima, K. Nakagawa, S. Tanaka, K. Tabata, R. Maysuse, K. Uchida, M. Hoshimoto, I. Hirata, and K. Katsu. 2002. Lactoferrin in whole gut lavage fluid as a marker for disease activity in inflammatory bowel disease: Comparison with other neutrophil-derived proteins. Am. J. Gastroenterology. 97:360-369.
7. Riley, L. W. 1995. Acute inflammatory diarrhea. In M. Blaser (ed.), P. Smith, J. Ravdin, H. Greenberg, and R. Guerrant, Infections of the Gastrointestinal Tract. Raven Press, New York, NY.
8. Sartor, R. B. 1995. Microbial agents in pathogenesis, differential diagnosis, and complications of inflammatory bowel disease. In M. Blaser (ed.), P. Smith, J. Ravdin, H. Greenberg, and R. Guerrant, Infections of the Gastrointestinal Tract. Raven Press, New York, NY.
9. 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. Gastroenterology. 91:927-934.
10. 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.
11. 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, pp. 44-48. American Society for Microbiology.

ASSISTANCE

If you have any questions regarding the use of this product, please contact Quidel's Technical Support Number at 1.800.874.1517 (in the U.S.) or 858.552.1100, Monday through Friday, from 7:00 a.m. to 5:00 p.m., Pacific Time. If outside the U.S., contact your local distributor, or technicalsupport@quidel.com.

CL91-345-01_EN_B (05/19)

Test Procedure Approval and Review Sheet

Prepared By:	
Date:	
Supervisor Review:	
Date:	
Laboratory Director or Designee Approval:	
Implementation Date:	
Supersedes Procedure Dated:	
Date Procedure Retired:	

Laboratory Director or Designee	Date Reviewed	Laboratory Director or Designee	Date Reviewed

QuickVue TLI Lactoferrin Test Verification Form

Account Name: _____

Address: _____

_____

Telephone: _____

QuickVue TLI
Lactoferrin Test Lot #/Exp: _____

Date: _____

Supervisor Signature: _____

Record the results from reference samples below.

Record the Sample #, the **QuickVue TLI Lactoferrin Test** results, Tester's Initials, and any comments. After the **QuickVue TLI Lactoferrin Test** results have been recorded (positive or negative) then record the Expected Results (positive or negative).

Sample #	Expected Results	**QuickVue TLI Lactoferrin Test** Result	Tester's Initials	Comments

QuickVue TLI Lactoferrin Verification Form (continued)

Sample #	Expected Results	**QuickVue TLI Lactoferrin Test** Result	Tester's Initials	Comments

Review: _____ Date: _____

Laboratory Director Review and Approval for Clinical Use: _____

Date: _____

QuickVue TLI Lactoferrin Test External Quality Control

There are two options for complying with CLIA's daily QC requirements for non-waived test systems under Section 493.1256 of the regulations:

- *Run two levels of external controls daily before patient testing OR*
- *Laboratories may develop and implement an IQCP for each non-waived test system.*

TECHLAB IQCP Support Documents may obtained upon request. The following listed conditions are also required as a minimum requirement:

External QC testing is recommended:

- When a new shipment of kits is received
- Additional tests can be performed with the controls to meet the requirements of local, state, and/or federal regulation and/or accrediting organizations

Date	**QuickVue TLI Lactoferrin Test** Kit Lot/Exp	Positive Ctrl Lot/Exp	Negative Ctrl (Diluent) Lot/Exp	Positive Control Result	Negative Control Result	Tester's Initials	Comments

Reviewed by: _____ Date: _____

QuickVue TLI Lactoferrin Test Internal Control Results and Patient Record

Lot Number _____ **Exp. Date** _____

Record the Date, Patient's Name, Patient Test Result, Internal Control Results and the Tester's initials.
Positive Internal Control = A red control line must be visible on the "C" side of the *Results Window* on every *Membrane Device* that is tested.
Negative Internal Control = The background in the result area will be clear to give a discernible result.

Date	Patient Name	Patient ID Number	Patient Results	Are the Internal Control Results Invalid or Valid?		Internal Controls Results		Comments	Tester's Initials
				Invalid	**Valid**	**+**	**–**		

Reviewed by: _____Date: _____

QuickVue TLI Lactoferrin Test Lot to Lot Comparisons

Name of Facility: _____

External Quality Controls are required to test a new lot of reagents.

- When a new shipment or new lot of kit is received
- When required by local, state, and/or federal regulations, accrediting groups, or your lab's Quality Control procedures

	CURRENT QuickVue TLI Lactoferrin Test In-Use Kit					**NEW QuickVue TLI Lactoferrin Test Kit**					
Date	**QuickVue TLI Lactoferrin Test** Kit Lot/Exp	Pos Control Lot/Exp	Neg Control Lot/Exp	Pos Control Result	Neg Control Result	**QuickVue TLI Lactoferrin Test** Kit Lot/Exp	Pos Control Lot/Exp	Neg Control Lot/Exp	Pos Control Result	Neg Result	Tech's Initials

Reviewed by: _____ Date: _____

Quality Assessment Review Form and Checklist

These forms are used for periodical review of the patient testing process. These should be filed with the quality assessment records.

Quality Assessment Activity	Comments	Date	Initials
Patient Test Management: Evaluate criteria for specimen submission, handling, and rejection; test results requisitions and reporting, accuracy and reliability of reports.			
Quality Control: Assess control data, errors in reporting results, and corrective actions taken with appropriate documentation records.			
Proficiency Testing: Review the effectiveness of corrective actions taken for unsatisfactory performance or failures.			
Comparison of Test Results: Review at least semi-annually comparative results for multiple methods, instruments, or site correlations when more than one procedure exists.			
Relationship of Patient Test Information to Test Results*:* Evaluate patient test reports for accuracy of patient information, test results, and normal ranges. Identify and evaluate results inconsistent with Patient's age, sex, diagnosis, and other test parameters.			
Personnel: Evaluate the effectiveness of policies and procedures for assuring employees' competence of testing and reporting test results.			
Communications: Evaluate documented problems and corrective actions that occur between the laboratory and the authorized individual who orders or receives the test result.			
Complaint Investigation: Evaluate documented complaints and corrective actions.			
Quality Assessment Reviews with Staff: Document discussion with Staff regarding identified problems and corrective actions during the QA review.			

Temperature Log

Equipment: _____

Name of Facility: _____

To be recorded at the beginning of each workday. Temperature Range: _____

Date	˚C	Initials	Adjustments	Date	˚C	Initials	Adjustments