h LACTOFERRIN CARD

For in *Vitro* diagnostic use only

Immunochromatographic card test for the detection of Human Lactoferrin in faeces

I.INTRODUCTION AND INTENDED USE

Lactoferrin (Lf) is a glycoprotein that is produced by neutrophils, mononuclear phagocytes and epithelial cells and is contained in the secretory fluids such as saliva and breast milk. Its function is to block bacterial growth by limiting the availability of iron and this effect is enhanced by the presence of specific secretory IgA antibodies directed against bacteria. Lf also has a bacteriocidal effect by causing direct damage to cell membranes in cooperation with lisozyme. When inflammation develops in the gastrointestinal tract, neutrophils and phagocytic cells migrate to the inflammatory focus and release the granules containing Lf. Lf is stable in faeces and is easily detected for immunochemical methods.

This marker is elevated in patients with inflammatory bowel disease. Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn disease (CD), represent a spectrum of diseases characterized by an idiopathic and chronic inflammation affecting the gastrointestinal (GI) tract. Pediatric and adult patients with IBD may present with a variety of clinical symptoms (including abdominal pain and diarrhea) that can be non-specific.

The h Lactoferrin Card is a rapid chromatographic immunoassay for the qualitative detection of human Lactoferrin in faecal samples that may reflect intestinal inflammation in inflammatory bowel disease (IBD).

II. PRINCIPLE OF THE TEST

The h Lactoferrin card is a non-invasive assay used as a way to differentiate patients with inflammatory (invasive bacterial infection, IBD, etc.) from those with noninflammatory (viral, toxigenic, etc.) gastrointestinal illness.

The h Lactoferrin card is a qualitative immunochromatographic assay for the determination of human lactoferrin in faecal samples. The membrane is pre-coated with antibodies on the test band (result region), against human lactoferrin.

During testing, the sample is allowed to react with the coloured conjugate (anti-human lactoferrin antibodies-red microspheres) pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate. A coloured band will be visible, depend on the lactoferrin content of the sample. This band is used to interpret the result.

The mixture continues to move across the membrane to the immobilized antibody placed in the control band region; this green coloured band always appears. The presence of this green band serves as verification that sufficient volume is added, that proper flow obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contains:

1. Lactoferrin-card (25 devices)
lactoferrin card device with a desiccant.
2. Extraction buffer tube (25 tubes)
3. Instruction for use (1)

Required materials (not supplied)

Specimen collection container
Disposable gloves and container
Timer

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- The kit is for in vitro diagnosis only.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Disposable gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another lot.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Discard the dilution buffer if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.

V. STORAGE

The test must remain in the sealed pouch until use and in a dry environment. The kit must not be frozen.

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the sealed pouch.

VI. SPECIMENS COLLECTION

The specimen should be transported in an airtight container and stored at +2℃ - +8℃ until tested. The specimen should be tested as soon possibile, but may be held up to 72 hours at +2℃ - +8℃ prior to testing. If testing cannot be performed within this time frame, specimens should be frozen immediately on receipt and stored frozen (≤ -20℃) until tested. Specimens may be frozen and thawed twice.

Note

Stool in transport media, on swabs, or mixed with preservatives is not appropriate for testing.

Mix stool as thoroughly as possible prior to pipetting.

Liquid or Semi-Solid Stools

Using a separate pipette (included with the kit) for each stool, draw stool of the sample itself. Dispense 10µL of each stool into a separate extraction tube. Mix carefully, then vortex 15 seconds.

Care should be taken when pipetting semi-solid stool. The addition of less than indicated of stool may cause a false-negative test. The addition of more than indicated of stool may cause invalid results due to restricted sample flow.

Formed / Solid Stools

Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen. Verify to transfer a small portion, to pick up a little sample (approx 10mg) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. Wait at least 3 minutes. Repeat the operations just to obtain a dark yellow-brown solution, if necessary. **The transfer of too little stool, or failure to mix and suspend the stool in extraction tube completely may result in a false-negative test results. Care should be taken to transfer no less and no**

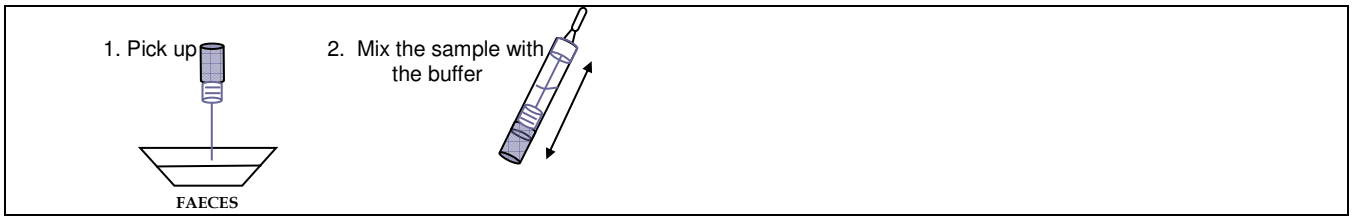
more than the amount indicated. The sample should be thoroughly mixed with a vortex before testing. The addition of excessive amount of stool may cause invalid results due to restricted sample flow.

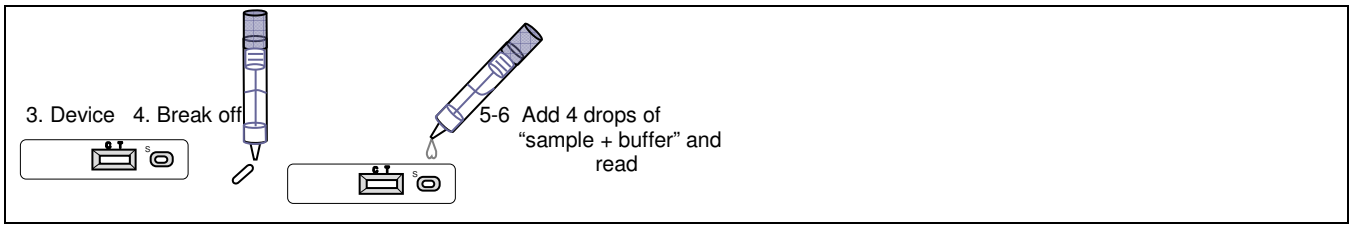
VII. TEST PROCEDURE

Test Procedure

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the Lactoferrin Card from its sealed pouch and use it as soon as possible. Place in a clean and flat surface.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial.
3. Use a separate device for each sample. Dispense 4 drops or 100 µL into the specimen well (S). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.





VIII. INTERPRETING THE RESULTS

Results are to be interpreted as follows, for each side:

POSITIVE: In addition to the Green control band, a Red band (lactoferrin test line) also appears in the site marked with the letter T. Interpretation: probably IBD (Inflammatory bowel disease).

NEGATIVE: Only one Green band appears across the central window in the site marked with the letter C. Interpretation: probably non active IBD (Inflammatory bowel disease).

INVALID: A total absence of the control coloured band (Green) regardless the appearance or not of the result line (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are likely the reasons for control line failure. Review the procedure and repeat the tests using a new test.

The intensity of the red coloured band in the result region (T) will vary depending on the concentration of human lactoferrin in the specimen. However, neither the quantitative value, nor the rate of increase in lactoferrin can be determined by this qualitative test.

IX. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A RED line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

X. PERFORMANCE

A. ExpectedValues

In infection bowel diseases, circulating neutrophils migrate into the infected tissues and release many kinds of granules.

Lactoferrin is associated with the secondary (specific) granules, which are released synchronously with other lysosomal proteins during phagocytosis. Thus, fecal lactoferrin is thought to be a marker of leukocyte activity in bowel infections.

The increase in fecal leukocytes suggests an inflammatory response to bacterial infection, including *Salmonella* species, *Shigella* species, *Campylobacter jejuni* and *Clostridium difficile*, while in a majority of viral infections, appears to be an invasive inflammatory process with little neutrophil migration.

B. Sensitivity- Specificity (Correlation)

A sample containing lactoferrin at concentration equal to or higher than 10ug hLf/g feces produces positive results when using the h Lactoferrin Card.

Different lactoferrin dilutions were tested directly in the extraction buffer or spiked in a negative stool sample in accordance with the kit instructions to determinate the detection limit of the test.

The detection of human lactoferrin with the h Lactoferrin Card test showed >99% of sensitivity compared to another commercial immunoassay.

C. Interference and cross-reactivity

The detection of human lactoferrin with the h Lactoferrin Card test showed 92% of specificity compared to another commercial immunoassay. The h Lactoferrin Card test is specific for human lactoferrin, showing no cross-reaction with bovine lactoferrin.

XI. LIMITS OF THE KIT

1. The test must be carried out within 2 hours of opening the sealed bag.
2. An excess of stool sample could result in wrong results (brown bands appear or absence of the control coloured band).
3. Stool from patients with active inflammatory bowel diseases that usually involve significant neutrophilic inflammation of the instestine, such as Crohn's disease and ulcerative colitis, would be positive for fecal lactoferrin. The h Lactoferrin Card could be sensitive for this diagnosis in patients with chronic diarrhea.

4. Positive results confirm the presence of human lactoferrin in fecal samples; nevertheless, it can be also due to several causes besides IBD. A positive result should be followed up with additional diagnostic procedures. Endoscopy and histology on biopsy specimens are the methods for detecting and quantifying bowel inflammation.
5. Negative results do not exclude inflammation, some diseases such as celiac disease and microscopic colitis polyps that involve mainly monocuclear inflammation.
6. Lactoferrin is a component of breast milk; the test will be positive in breast fed children and should not be used to evaluate neonates receiving breast milk.

XII. REFERENCES

- Amemoto K. et al. Clinical evaluation of fecal lactoferrin and α-1-antitrypsin in pediatric gastrointestinal infections, Pathophysiology. Vol 3; 1996, p. 87-90.

- Walker, T.R. et al. Fecal Lactoferrin is a Sensitive and Specific Marker of Disease Activity in Children and Young Adults with Inflammatory Bowel Disease. J. Pediatr Gastroenterol. Nutr. April 2007; Vol 44, No 4, p. 414-422.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (25 tests)
Card
Extraction Buffer tube
Instruction for use

COD. VT81600
25 devices
25 items x 1.0 mL
1 item

EDMA Code 12 01 03 05

CE IVD