Lactoferrin Blister Rapid Test

Cat. No.:DTS521
Pkg.Size:

Intended use

The Lactoferrin Blister Rapid Test 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).

General Description

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 Lactoferrin Blister Rapid Test 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.

Principle Of The Test

The Lactoferrin Blister Rapid Test 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 red coloured band always appears. The presence of this red band serves as verification that sufficient volume is added, that proper flow obtained and as an internal control for the reagents.

Reagents And Materials Provided

1. Lactoferrin Blister Rapid Tests.
2. Instructions for use.
3. Specimen collection vial with buffer.

Materials Required But Not Supplied

1. Specimen collection container.

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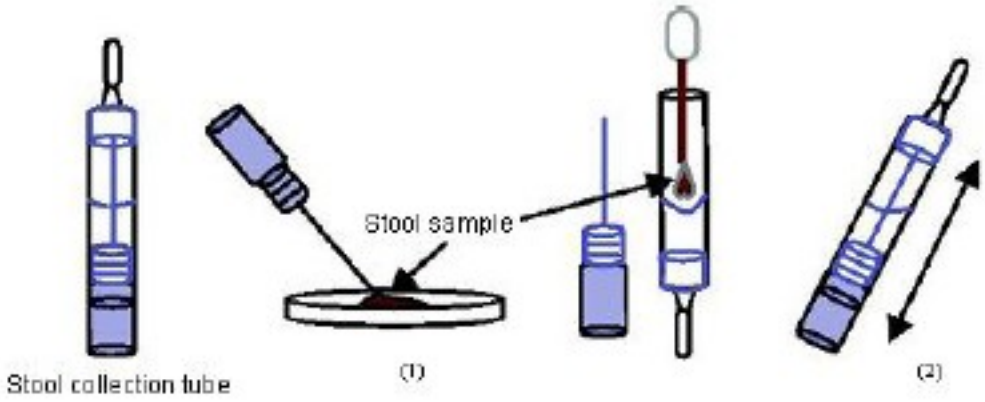
2. Disposable gloves.
3. Timer.

Storage

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30℃/36-86℉). The test is stable through the expiration date printed on the sealed pouch.
The test must remain in the sealed pouch until use. Do not freeze.

Specimen Collection And Handling

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-4℃/36-40℉) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at –20°C/4oF. In this case, the sample will be totally thawed, and brought to room temperature before testing.
Figure 1.



Assay Steps

Allow the tests, stool samples and buffer to reach to room temperature (15-30℃/59-86℉) prior to testing. Do not open the pack until ready to perform the assay. There are two possibilities for performing the test:
A: Using the blister test single pack as a Card test:
1. Cut the blister to obtain a test single pack, hold the non sealed side and open it peeling off the upper foil. Don't remove the test from the blister cavity and use it as soon as possible.
2. Shake the specimen collection tube to assure good sample dispersion. Cut the end of the top.
3. Place blister test single pack horizontally and identity it. Dispense 5 drops of sample+buffer on the white end of the test. Start the timer. Read the result at 5 minutes after dispensing the sample.
B: Using the blister test single pack as a strip test: By immersion:
1. Cut the blister to obtain a test single pack, hold the non sealed side and open it peeling off the upper foil.
2. Shake the specimen collection vial to assure good sample dispersion. Cut the end of the top.
3. Dispense 5 drops of sample+buffer in an identified vial and leave the test strip to stand vertically in the vial , taking care of not surpassing the limit of immersion indicated with the arrows. Start the timer. Read the result at 10 minutes.

Quality Control

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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.

Reference Values

Expected Values:

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.

Interpretation of Results

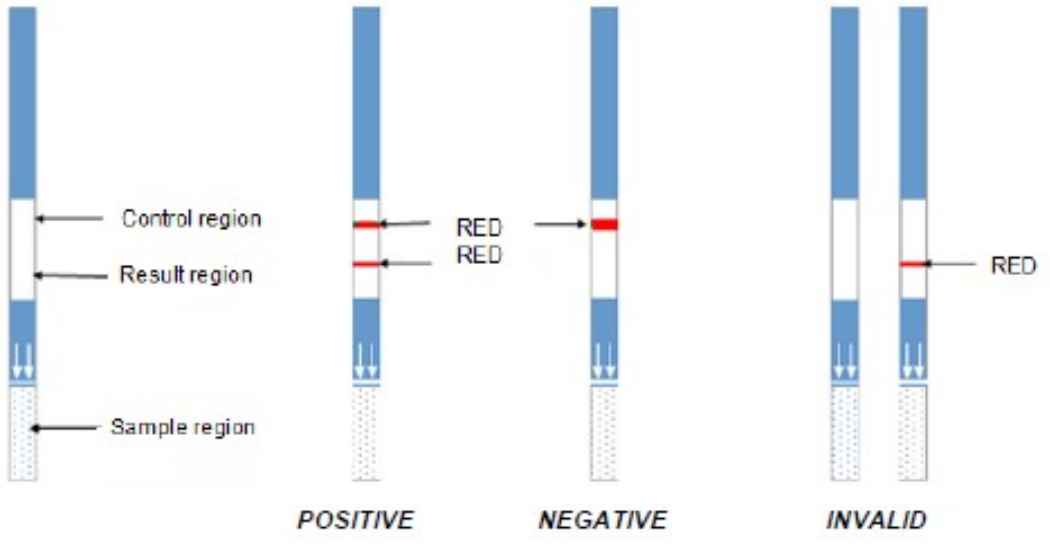
Positive: In addition to the RED control band, a RED band (lactoferrin test line) also appears in the site marked with the letter T (result region). Interpretation: probably IBD (Inflammatory bowel disease).

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

Invalid: A total absence of the control coloured band (RED) 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. If the problem persists, discontinue using the test kit and contact you local distributor.

Note: 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.

Figure 2.



Sensitivity

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A sample containing lactoferrin at concentration equal to or higher than 10ug hLf/g feces produces positive results when using the Lactoferrin Blister Rapid Test.
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 Lactoferrin Blister Rapid Test showed > 99% of sensitivity compared to another commercial immunoassay.

Specificity

The detection of human lactoferrin with the Lactoferrin Blister Rapid Test showed 99% of specificity compared to another commercial immunoassay.
The Lactoferrin Blister Rapid Test is specific for human lactoferrin, showing no cross-reaction with bovine lactoferrin.

Precautions

1. Do not use after expiration date.
2. The test should remain in the sealed pouch until use.
3. Do not use the test if pouch is damaged.
4. Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
5. All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
6. The test should be discarded in a proper biohazard container after testing.
7. The test must be carried out within 2 hours of opening the sealed bag.

Limitations

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 Lactoferrin Blister Rapid Test 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 sprue 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.

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