# immunocard STAT!® CGE

## One-step immunochromatographic test for the qualitative detection of *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica* antigens in human stool samples

REF 751420 IVD

### INTENDED USE

Immuno*Card* STAT! CGE is a rapid in vitro qualitative immunoassay that simultaneously detects and distinguishes between *Cryptosporidium parvum*, *Giardia lamblia and Entamoeba histolytica* antigens in human stool. This assay is used as an aid in the diagnosis of related parasitic infections.

### SUMMARY AND EXPLANATION OF THE TEST

Cryptosporidiosis is a protozoal infection caused by *Cryptosporidium parvum* in humans. Typically, it is an acute short-term infection, but can become severe and non-resolving in children and immunocompromised individuals such as AIDS patients. It may also be asymptomatic. In tropical developing countries, the parasite is often endemic and causes diarrhoea epidemics among children. With immunocompetent patients, the disease manifests itself as self-healing gastroenteritis.

Giardiasis in humans is caused by the protozoan parasite *Giardia lamblia*. This organism is involved in 25% of the cases of gastrointestinal disease and may present asymptomatically. *Giardia lamblia* has become an important cause of chronic diarrhoea, particularly regarding travel medicine.

*Entamoeba histolytica* is the protozoan parasite responsible for dysentery and amebiasis. It is the third leading cause of morbidity and mortality due to parasitic disease in humans after malaria and schistosomiasis, and is estimated to be responsible for between 50000 and 100000 deaths every year. The disease may manifest itself as an acute, chronic or as an asymptomatic infection.

All these parasites present a simple life cycle that usually consists of an infective cyst stage and a multiplication trophozoite stage. Transmission of these infections occurs via ingestion of cysts, most often via food or water contaminated with human faecal matter.

In the case of *Entamoeba*, a new understanding of this organism has led to the recognition that two species actually exist within what was previously known as *E. histolytica.* Of these two organisms *E. histolytica* is the pathogenic species causing all invasive disease while the other, *E. dispar* is non pathogenic, as it is not capable of invading tissue. The two species are morphologically identical, so although diagnosis is usually performed by microscopic examination, it cannot differentiate them. Other techniques are required to detect specific antigens of each species for an accurate diagnosis and to prevent unnecessary or inappropriate chemotherapy.

The Immuno*Card* STAT! CGE is based on the immunological capture of coloured microparticles as they move along a membrane on which the monoclonal antibody has been immobilized.

## BIOLOGICAL PRINCIPLES

Immuno*Card* STAT! CGE test uses specific monoclonal antibodies against *Cryptosporidium parvum, Giardia lamblia* and *Entamoeba histolytica* that detect all forms of the parasites during their life cycle.

The test is based on the use of three types of microspheres: blue microspheres covalently linked to a monoclonal anti-*Cryptosporidium parvum* antibody, red microspheres covalently linked to a monoclonal anti-*Giardia lamblia* antibody, green microspheres covalently linked to a monoclonal anti-*Entamoeba histolytica* antibody as well as purple microspheres used as test control.

The parasites present in stool samples react with the latex particles which are coated with specific monoclonal antibodies against the antigen. This *latex particles/antibodies/parasite* complex migrates through a chromatographic process towards the reaction area. In this area, anti-*Cryptosporidium,* anti-*Giardia* and anti-*Entamoeba* antibodies that react with the *latex particles/antibodies/parasite* complex are present. This reaction leads to the appearance of a blue and/or red and/or green lines. These lines are used to interpret the result, following a ten-minute room-temperature incubation.

## REAGENTS/MATERIALS PROVIDED

***The maximum number of tests obtained from this test kit is listed on the outer box.***

- **Immuno*Card* STAT! CGE Device:** Individually foil pouched device containing immobilized monoclonal antibody against the *Cryptosporidium parvum, Giardia lamblia* and *Entamoeba histolytica* antigens.

- **Sample Diluent/Negative Control Vial:** a buffered diluent containing 0.095% sodium azide as a preservative. The reagent is supplied in a plastic dropper vial. Use as supplied.

## MATERIALS NOT PROVIDED

1. Applicators for sample collection (solid or liquid)
2. Vortex mixer
3. Test tubes or 1.5mL capped microtubes
4. Centrifuge (optional)
5. Appropriate micropipettes
6. Pipette tips
7. Timer
8. Disposable gloves
9. Positive Control (Product Code: 751403)

## PRECAUTIONS

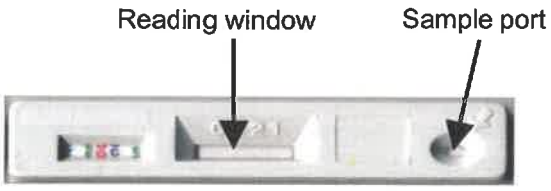
1. All reagents are for in vitro diagnostic use only.
2. Directions should be read and followed carefully.
3. Reagent concentration, incubation times and temperatures (20–30 C) have been optimized for sensitivity and specificity. Best results are obtained by adhering to these specifications. Once the assay has been started, complete all subsequent steps without interruption.
4. All reagents should be gently mixed at 20–30 C before use.
5. Do not interchange reagents from different lot numbers.
6. Do not use the Sample Diluent/Negative Control if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
7. Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use test devices from pouches that have holes in the foil or where the pouch has not been completely sealed. False reactions may result if test components are improperly stored.
8. Do not use kit or components beyond their assigned expiration dates.

9. Patient specimens may contain infectious agents and should be handled by properly trained personnel and disposed of as potential biohazards. Wear disposable gloves while handling patient specimens and performing the test procedure.
10. The Sample Diluent/Negative Control vial contains the preservative sodium azide which is a skin irritant. Avoid contact. Disposal of reagents containing sodium azide into drains consisting of lead or copper plumbing can result in the formation of explosive metal oxides. Eliminate the build-up of oxides by flushing drains with large volumes of water during disposal.
11. Do not use the Sample Diluent/Negative Control if signs of contamination or precipitation are observed.
12. Stool must be mixed thoroughly, regardless of consistency, to ensure a representative sample prior to pipetting.
13. It is important to take the appropriate amount of stool sample and reagent, Over-inoculation of stool into the Sample Diluent may cause clogging and not allow the sample migration to the Test Zones.
14. Do not use the test if a coloured line appears in the result area before its use.
15. Centrifugation: In order to ensure adequate chromatography and optimal results, it is recommended to centrifuge the 1.5 mL microtubes containing the diluted sample prior to transferring the specific quantity of supernatant. This is particularly true in the case of solid stool samples, as the greater number of suspended particles can interfere with the chromatography. However, if centrifugation is not possible, wait some minutes to settle solid particles before performing the assay.

**HAZARD and PRECAUTIONARY STATEMENTS**
There are no known hazards associated with this product.

**SHELF LIFE AND STORAGE**
The expiration date is indicated on the kit label. Store the kit at 2-30 C when not in use.

**PROCEDURAL NOTES**
The Immuno*Card* STAT! CGE Test Card is shown in the diagram below:




Batch processing of samples or controls is possible provided the correct incubation time is maintained for each device.

The Control zone of each device is a procedural control to ensure that the sample has migrated sufficiently in the device to permit a valid test result that can be read.

**SPECIMEN COLLECTION**
For best results, specimens should be collected as soon as possible after onset of symptoms. Stool samples should be received in an airtight container and may be stored up to 48 hours at 2-8 C prior to testing. Freeze specimens immediately upon receipt if testing cannot be completed within 48 hours.

Avoid freezing and thawing the samples several times. Do not use stool in transport medium such as 10% formalin, SAF, PVA, Ecofix®, etc., because they interfere with the test.

## SPECIMEN PREPARATION

1. Bring specimens to 20-30 C. Mix stool as thoroughly as possible prior to pipetting.
2. Mix Sample Diluent/Negative Control prior to use. Using the dropper assembly provided with the Sample Diluent/Negative Control, add **1.0 mL** of Sample Diluent to a test tube.
3. Add a small portion of approximately **5-6 mm size** (50 mg) with a swab, a wooden applicator or a bacteriological loop.

**For liquid or semi-solid stools**, add **100µL** of stool using an appropriate pipette.
Vortex for a minimum of 15 seconds.

4. Wait 3-5 minutes until the solid particles settle at the bottom of the tube or centrifuge for 5 minutes at 700 xg (approximately 3000 rpm in a benchtop centrifuge).

## TEST PROCEDURE

1. Remove the Immuno*Card* STAT! CGE test device from its foil pouch and label with the patient identification.
2. Add **125 µL** of the prepared specimen to the sample port of the device. Avoid as much as possible transfer of solid particles.
3. Incubate the test at 19-27 C for **10 minutes**.
4. Read the results within **1 minute** of the end of incubation.

## INTERPRETATION OF RESULTS

Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.

***NEGATIVE:***
Only a single **PURPLE** colored band appears at the Control Line marked 'C' on the device frame. No other bands are present.

***POSITIVE:***
***Cryptosporidium*:**
In addition to the **PURPLE** Control band, a **BLUE** band appears at position '1' on the device frame, in the interpretation window. A **BLUE** line, even very weak, is indicative of the presence of *Cryptosporidium* antigens.

***Giardia lamblia*:**
In addition to the **PURPLE** Control band, a **RED/PINK** band appears at position '2' on the device frame, in the interpretation window. A **RED/PINK** line, even very weak, is indicative of the presence of *Giardia Lamblia* antigens.

***Entamoeba histolytica*:**
In addition to the **PURPLE** Control band, a **GREEN** band appears at position '3' on the device frame in the interpretation window. A **GREEN** line, even very weak, is indicative of the presence of *Entamoeba histolytica* antigens.

***INVALID***:
- No **PURPLE** band at the designated position for the Control Line. The test is invalid as the absence of a control band indicates the test procedure was performed improperly or that deterioration of reagents has occurred.
- Colored band appearing at one or more Test Line positions of the device after the defined incubation limit has no diagnostic value.
- Band of any color other than the defined ones.

| | | | | |
|---|---|---|---|---|
|  |  |  |  |  |
| **Negative** | *Crypto* | *Giardia* | *Entamoeba* | *Crypto/ Giardia/ Entamoeba* |
| | **Positives** | | | |

**QUALITY CONTROL**

***This test should be performed per applicable local, state, or federal regulations or accrediting agencies.***

A **PURPLE** band appearing at the control line serves as internal positive control and indicates the test has been performed correctly, that sample was added, that it flowed properly, and that the test reagents were active at the time of use.

**WARNING:** Control of the Immuno*Card* STAT! CGE device reactivity with the Positive Control reagent (Meridian Product Catalog# 751403) and/or with known Positive samples is recommended to ensure that the data obtained are correct.

The number of additional tests performed with the external controls will be determined by the requirements of local, state or national regulations or accrediting agencies. **If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.**

**LIMITATIONS OF THE PROCEDURE**

1. The test should be used only for detecting *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica* antigens in human stool.
2. This is a qualitative test and no quantitative interpretation of the results should be made with respect to the intensity of the positive line.
3. Over 200 samples were evaluated to ensure proper test performance. The correlation of results with other techniques (ELISA) was good. However, interferences in the test performance cannot be excluded.

4. If there is an excessive sample amount, **BROWN LINES** may appear instead of the blue, red, green and purple ones, or test and control lines may be absent because chromatography is not correctly conducted. The BROWN LINES have no diagnostic value. If this happens, repeat the test with a smaller amount of stool sample.
5. Due to the homology between *E. histolytica* and *E. dispar*, certain cross-reaction may occur between these two species but it is expected to be less than a 5%. No cross-reaction with other substances has been observed during test evaluation.
6. A negative result does not totally exclude the possibility of *Cryptosporidium parvum, Giardia lamblia* and/or *Entamoeba histolytica* infection. The significance of the results should be evaluated in relation to the patient's clinical symptoms.
7. The analysis of certain samples can produce lines of IMPRECISE COLOUR, mostly corresponding to negative samples. The test should be repeated if lines of indeterminate colour appear. If the same result is obtained again, the analysis should be performed by another analytic method.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

The Meridian immunochromatographic test designed for *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica* detection was externally and internally evaluated against different techniques (Microscopy, ELISA and/or PCR). The results were as follows:

*Cryptosporidium parvum*:

| Meridian Crypto test | | | |
|---|---|---|---|
| | | Microscopy | Commercial ELISA Crypto |
| ImmunoCard STAT! CGE | Sensitivity | 80.0% | 79.3% |
| | 95% CI | 71.1-87.2 | 72.0-85.5 |
| | Specificity | 96.9% | 99.5% |
| | 95% CI | 95.0-98.2 | 98.5-99.9 |

*Giardia lamblia*:

| Meridian Giardia test | | | |
|---|---|---|---|
| | | Microscopy | Commercial ELISA Giardia |
| ImmunoCard STAT! CGE | Sensitivity | 92.4% | 93.8% |
| | 95% CI | 87.3-95.9 | 89.2-96.9 |
| | Specificity | 98.6% | 98.9% |
| | 95% CI | 97.3-99.3 | 97.6-99.6 |

*Entamoeba histolytica*:

| Meridian Entamoeba test | | Commercial ELISA Entamoeba | E. histolytica PCR |
|---|---|---|---|
| ImmunoCard STAT! CGE | Sensitivity | 82.4% | 75.0% |
| | 95% CI | 71.2-90.5 | 53.3-90.2 |
| | Specificity | 96.4% | 89.8% |
| | 95% CI | 94.1-97.9 | 86.4-92.6 |

**REPRODUCIBILITY**

**INTRA-ASSAY PRECISION**

Triplicates for each concentration of the sensitivity curve were assayed with one lot of the product, obtaining the same results.

**INTER-DAY PRECISION**

Using one product lot, triplicates of the sensitivity curve were performed over 10 consecutive days by the same person. Only one difference, of less than one 1:2 dilution, was found, which is acceptable and tolerable for a qualitative immunochromatographic technique.

**INTER-LABORATORY PRECISION**

Five different laboratory technicians assayed the same samples, obtaining high precision and concordance. Only a difference of one 1:2 dilution was found, which is acceptable and tolerable for a qualitative immunochromatographic technique.

**INTER-LOT PRECISION**

A sensitivity curve was performed using three product lots. The analyses were performed by the same person on the same day. Only one difference, of less than one 1:2 dilution, was found, which is acceptable and tolerable for a qualitative immunochromatographic technique.

**ASSAY SPECIFICITY**

The microorganisms indicated below did not cause any interference in the results.

**Bacteria/Parasites/Viruses**

| | | |
|---|---|---|
| *Entamoeba coli* | *Iodamoeba bütschlii* | *Hymenolepsis nana* |
| *Giardia lamblia* | *Cryptosporidium parvum* | *Helicobacter pylori* |
| *Blastocystis hominis* | *Endolimax nana* | *Clostridium difficile* |
| *Entamoeba hartmannii* | Rotavirus | Adenovirus |

**TESTS FOR INTERFERING SUBSTANCES**

The substances indicated in the table, at the specified concentration, did not interfere with the results. Only Ibuprofen* at higher concentration could interfere in the detection of a very low Giardia positive sample. A single lot was used to conduct the study.

| | |
|---|---|
| Metronidazole | 1.2 mg/mL |
| Racecadotril | 0.72 mg/mL |
| Loperamide | 24 µg/mL |
| Braun Atropine | 1.68 µg/mL |
| Cimetidine | 1.92 mg/mL |
| Omeprazole | 0.144 mg/mL |
| Neomycin | 6.62 mg/mL |
| Ampicillin | 7.2 mg/mL |
| Ibuprofen* | 5.76 mg/mL |
| Acetylsalicylic Acid | 4.8 mg/mL |
| Sucrose | 8 mg/mL |
| Blood | 10% (v/v) |

**ITALIANO**

# immunocard STAT!® CGE

## Test immunocromatografico rapido per la rilevazione qualitativa degli antigeni di *Cryptosporidium parvum*, *Giardia lamblia* ed *Entamoeba histolytica* in campioni di feci umane

REF **751420**

IVD

### FINALITÀ D'USO

Immuno*Card* STAT! CGE è un test immunologico qualitativo per la rilevazione simultanea e distinta di antigeni di *Cryptosporidium parvum*, *Giardia lamblia* ed *Entamoeba histolytica* in campioni di feci umane. Questo test è da utilizzarsi come ausilio nella diagnosi di infezioni parassitarie correlate.

### SOMMARIO E SPIEGAZIONE DEL TEST

Nell'uomo, la Criptosporidiosi è un'infezione dovuta a un protozoo, il *Cryptosporidium parvum*. Di solito, si tratta di un'infezione acuta e a breve termine, ma può diventare severa e durare per tutta la vita nei bambini oppure in individui il cui sistema immunitario è già compromesso, come per esempio nei pazienti affetti da AIDS. Può anche essere asintomatica. Nei paesi tropicali in via di sviluppo, il parassita spesso è endemico e causa epidemie di diarrea tra i bambini. In pazienti immunocompetenti, la malattia si manifesta come una gastroenterite autolimitante.