Crypto Strip

Quick Test

Code 339 Crypto Kit
25 dipsticks
15 ml vial dilution buffer

IVD
Store at 4 - 30ºC.

CYPRESS DIAGNOSTICS

CE

Immunochromatographic test for the detection of Cryptosporidium parvum oocysts in faeces.

Introduction

Cryptosporidiosis is a leading cause of persistent diarrhoea in developing countries due to the presence *Cryptosporidium parvum* in the gastrointestinal tract. This parasite is recognised as a highly infectious enteric pathogen and infective stage is transmitted by the faecal-oral route.
It is also a serious opportunistic pathogen in immunocompromised individuals without effective therapeutic treatments. Symptoms of cryptosporidiosis include watery diarrhoea, stomach cramps, weight loss, nausea and sometimes fever. In industrialised countries, 2 to 2.5 % of persons admitted to hospital and suffering of diarrhoea eliminate oocysts.
In AIDS patients, 10 % of individuals are suffering from chronic cryptosporidiosis and this figure rises up to 40 % in some undeveloped countries.
Diagnosis of *C. parvum* is carried out by using either acid-fast (modified Ziehl-Neelsen method) or immunofluorescence staining on unconcentrated faecal smears.
Several enzyme-linked immunosorbant assays are also available for detection of specific cryptosporidial oocysts antigens. New methods involving PCR may help to detect *Cryptosporidium spp* in water supplies or asymptomatic carriers. All these methods are sensitive but also time-consuming and should be performed by experimented operators.
Cypress has developed an immunochromatographic test that enables to detect *C. parvum* oocyst antigens in unconcentrated stool within 15 minutes.

Principle of the test

The test uses a new homogenous immunochromatographic system with gold particules. It is a simple-to-use test that only needs the dilution of the faecal sample with the supplied ready-to-use dilution buffer.
Specificity is ensured by using a monoclonal antibody directed against specific membrane antigens of *Cryptosporidium parvum* oocysts. These reagents are used conjugated with gold particules and coated on the nitrocellulose.
Liquid sample and gold conjugate both migrate by capillary action. Upon reaching the anti-Cryptosporidium monoclonal reagent, any Cryptosporidium oocyst present in the sample will be blocked and an immunoreaction will appear as a red line. The solution continues to migrate up the strip, on reaching a second reagent (an anti-chicken IgY), that binds a control conjugate, thereby producing a second red line. This second line indicates that the sample has migrated correctly and that the chromatography has developed without hindrance. The rear line appears with both positive and negative samples. If this line does not develop, the test is invalid.

Reagent composition

Each kit contains:
- Individual Crypto-Strip strips (25) : These strips come in a bottle with a desiccant.
- Dilution buffer (15 ml): Saline solution buffered to pH 7.5 with Tris and containing NaN3 (<0.1%), a detergent, and charged proteins.
- Instruction for use (1)

Required materials (not supplied) :

- 3 or 5 ml test tubes
- Inoculation loops for taking the faecal samples

Special precautions

- The Crypto-Strip is for in vitro diagnostic only. For professional use only.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Dispose of gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another kit.
- The bottle containing the sensitized strips must be recapped as soon as the necessary number of strips for the operation has been removed, since the strips are sensitive to humidity. Make sure that the desiccant sachet is present.
- Discard the buffer solution if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life dates or if the reagents are stored under inappropriate conditions.

Storage

- An unopened Adeno-Strip kit may be kept between 4 and 37°C and used until the shelf-life date on the packaging.
- The strips remain stable for 15 weeks after the bottle is opened if they are kept at between 4 and 37°C and in a dry environment.
- The Crypto-Strip kit must not be frozen.

Samples

The stool specimens must be tested as soon after they are collected. If necessary, they may be stored at 2-8°C for 1 week or -20°C for longer periods of time.
Make sure that specimens are not treated with solutions containing formaldehyde or its derivatives.

Procedure

Preparations:

Allow kit components, in unopened packaging, and samples to reach room temperature (15-30°C) before proceeding with the test.
Once opened, run the test immediately. Write the patient's name or specimen number on the test tube. Place the marked test tubes in a rack.

Instructions:

1. Add 0.5 ml or 15 drops of the dilution buffer solution to each tube.
2. Dip the inoculating loop containing the stool sample into the tube. The dilution ratio must be at the most 4% w/v. For liquid samples take 2 loops of 10 µl, for solid samples take 1 loop.
3. Vortex the solution to homogenize. The entire stool sample must be in suspension.
4. Discard the inoculating loop and immerse the sensitized strip in the direction indicated by the arrow. To avoid diluting the colloidal gold conjugate in the solution, take care not to immerse the strip above the line placed under the arrow.
5. Results must be read on wet strips after 15 minutes incubation. Positive results may be reported sooner the moment the test and control lines become visible. Do not take the appearance of new lines into account after the reaction time is passed.

Reading and interpretation

The results are to be interpreted as follows:

Negative:	1 line: Only a reddish-purple Control line (upper line) appears. No other line is present.
Positive:	2 lines: In addition to the Control line, a reddish-purple Test line appears. The intensity of the test line may vary according to the quantity of antigens found in the sample. Even a weak signal must be regarded as a positive result.
Invalid:	The absence of a control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.

After drying, a very slight shadow may appear at test line. It should not be regarded as a positive result.

Quality control

In accordance with Good Laboratory Practices, we recommend checking the test's performance regularly in line with the laboratory's requirements.

Limitations:

The test is qualitative and cannot predict the quantity of antigens present in sample.
A positive test does not rule out the possibility that other pathogens may be present. The Crypto Strip is an acute-phase screening test. Stool samples that are collected after this phase may contain antigen titres below the reagent's sensitivity treshold.
As with all diagnostic tests, all results must be considered with other clinical information available to the physician.

Performances:

Detectability

The Crypto-Strip limit of detection for cryptosporidium parvum is 50-100 oocysts in 100 µl of faeces.

Sensitivity – Specificity (Correlation):

An evaluation has been conducted on 100 stool samples in comparison with an immune enzymatic rapid test. Results of these tests are:

Sensitivity = 95,7% (45/47) Positive Predictive Value = 100% (45/45)
Specificity = 100 % (53/53) Negative Predictive Value = 96,4 % (53/55)
Accuracy = 98 % (98/100)

Reproducibility:

Intra-batch:
Cryptosporidium positive samples and dilution buffer were tested fifteen times by the same batch of Crypto-Strip kits.
The results were correct in 100% of the cases.
Inter-batch:
Cryptosporidium positive samples and dilution buffer were tested on three different batches of Crypto-Strip kits.
The results were correct in 100% of the cases.

Interference:

Tests for cross-reactivity to the following pathogens were conducted and found to be negative :
Salmonella typhimurium, Coronavirus, Entamoeba histolytica, Entamoeba dispar, several E. Coli strains (including E. Coli O157:H7 and E. Coli c600-933W), Rotavirus, adenovirus, E. Coli F5, Salmonella enteritidis, Giardia lambia, Giardia muris.
Tests for cross-reactivity has been tested on Staphylococcus aureus and found positive at high bacteria concentrations.

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