**INTENDED USE:**

The *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK* test is a rapid membrane enzyme immunoassay for the simultaneous qualitative detection and differentiation of *Giardia* cyst antigen and *Cryptosporidium* oocyst antigen. It is intended for use with human fecal specimens from patients with gastrointestinal symptoms to aid in the diagnosis of *Giardia* and/or *Cryptosporidium* gastrointestinal infection. The test results should be considered in conjunction with the patient history.

**PRINCIPLE:**

The *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK* test uses monoclonal and polyclonal antibodies to cell-surface antigens of the organisms. The device contains a *Reaction Window* with three vertical lines of immobilized antibodies. The *Giardia* test line (“Giar”) contains mouse monoclonal antibodies against *Giardia*. The Crypto test line (“Cryp”) contains mouse monoclonal antibodies against *Cryptosporidium*. The control line (“C”) is a dotted line that contains anti-horseradish peroxidase (HRP) antibodies. The *Conjugate* consists of polyclonal antibodies coupled to horseradish peroxidase. To perform the test, the sample is added to a tube containing a mixture of *Diluent* and *Conjugate*. The diluted sample-conjugate mixture is added to the *Sample Well* and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, cyst and/or oocyst antigens in the sample bind the antibody-peroxidase conjugates. The antigen-antibodyconjugate complexes migrate through a fi lter pad to a membrane where they are captured by the immobilized *Giardia* and/or *Cryptosporidium*-specifi c antibodies in the test lines. The *Reaction Window* is subsequently washed with *Wash Buffer*, followed by the addition of *Substrate*. After a 10 minute incubation period, the reaction is examined visually for the appearance of a vertical blue line on either side of the *Reaction Window*. A blue line indicates a positive test. A positive “control” reaction, indicated by a vertical dotted blue line under the “C” portion of the *Reaction Window*, confi rms that the test is working properly and the results are valid.

**MATERIALS AND EQUIPMENT:**

**Materials Provided:**

Alere Quik Chek test kit – Membrane devices, Diluent, Wash buffer, Substrate, Conjugate,

 Positive Control

 Disposable plastic pipettes – graduated at 25µL, 100 µL, 200 µL, 300 µL, 400 µL, and 500 µL

**Materials and Equipment Not Provided:**

Small plastic test tubes

 Timer

 Disposable gloves

 Applicator sticks

 Vortex mixer

 Pipettor and tips

**SHELF LIFE AND STORAGE**

The expiration date of the kit is given on the label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2° and 8°C.

**PRECAUTIONS**

1. Each component in the kit should be inspected for any signs of leakage. Upon arrival, inspect the kit to ensure that

 components are not frozen or warm to the touch due to improper shipping conditions.

2. The Substrate reagent should be colorless. If the Substrate reagent changes to a dark blue/violet color, discard and call

 technical services for replacement.

3. Reagents from different kits should not be mixed. Do not use a kit past the expiration date.

4. Caps, tips, and dropper assemblies are color-coded; do NOT mix or interchange!

5. Bring all components to ROOM TEMPERATURE BEFORE USE!

6. The pouch containing the Membrane Device should be at room temperature before opening. Keep the Membrane

 Devices dry before use.

7. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume.

8. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of

 reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.

9. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions

 may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.

10. Be attentive to the total assay time when testing more than one fecal specimen. Add *Diluent* first, and then add the

 *Conjugate* to each tube of *Diluent*. Then add specimen to the tube of *Diluent/Conjugate*. Thoroughly mix all of the

 diluted specimens, and transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted

 sample-conjugate mixture has been transferred to the *Membrane Device*.

11. Use fresh fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose

 activity due to freezing and thawing. If using frozen specimens, thaw at room temperature.

12. This test has been shown to be compatible with specimens preserved in 10% formalin, sodium acetate formalin, and

 transport media such as Cary Blair or C&S when following the “Sample Preparation” guidelines listed in this Package

 Insert. However, the test has not been proven compatible with other preservatives and transport media.

13. Specimens and *Membrane Devices* should be handled and disposed of as potential biohazards after use. Wear

 disposable gloves when doing the test.

17. Fecal specimens may contain potentially infectious agents and should be handled at “Biosafety Level 2” as

 recommended in the CDC/NIH Manual, “Biosafety in Microbiological and Biomedical Laboratories.”

**SPECIMEN:**

 **Acceptable Specimens**

Fresh, untreated stool samples stored at 2-8⁰C and less than 72 hours old

 Specimens preserved in 10% Formalin

 Note:Specimenscollected in Cary Blair or C&Smay be used for testing, but positives cannot be referred to the

 Minnesota Department of Health for confirmation of results.

 **Unacceptable Specimens**

Fecal specimens collected in PVA

 Fecal specimens collected in EcoFix

 Unrefrigerated fresh untreated fecal specimens

 Fresh untreated fecal specimens greater than 72 hours old that are not frozen

 Non-fecal specimens – duodenal wash or aspirate

 Concentrated fecal specimens

 **Test order Code: GIACRY**

**COLLECTION AND HANDLING OF FECAL SPECIMENS**

 **Fecal specimens should be collected in clean, leak-proof containers**.

1. Fresh, untreated specimens should be stored between 2° and 8°C. Test fresh specimens that are less than 72 hours

old, whenever possible. Store fresh specimens frozen (≤ -10°C) for up to 90 days if the test cannot be performed within 72 hours of collection, but note that freezing and thawing of the specimen may result in loss of activity due to degradation of the antigen. Avoid multiple freeze-thaw cycles. If using frozen specimens, thaw at room temperature.

1. Make sure that specimens are thoroughly mixed PRIOR to performing the assay.
2. Storing fecal specimens in the *Diluent* is NOT recommended.

**Collection of fecal specimens in transport device – 10% Formalin**

1. An area of the stool that is bloody, slimy, or watery should be selected with the collection spoon provided. For formed stools, material should be removed from the sides end or middle of the bolus.
2. Sufficient stool is added to the container to bring the liquid up to the “Fill to here” line. This requires approximately

 5 ml of stool or 1:4 dilution (1 part stool, 3 parts 10% formalin)

1. Cap and then shake the vial to insure the specimen is adequately mixed.

**SAMPLE PREPARATION**

1. Bring all reagents and the required number of devices to room temperature before use.
2. Set up and label one small test tube for each specimen and optional external controls as necessary
3. Mix all specimens thoroughly regardless of consistency – it is essential that the specimens be evenly suspended before transferring
4. **For fresh fecal specimens**:

Using the gray graduated dropper assembly, add 500 μL (2nd graduation from the tip) Diluent to each tube.

**For specimens in fixative or transport media:**

Using the gray graduated dropper assembly, add 400 μL of Diluent to the tube.

**For exernal controls:**

Add 400 μL of Diluent to the tube.

1. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample – the pipettes have raised graduations at 25 μL, 100 μL, 200 μL, 300 μL, 400 μL, and 500 μL. 
2. **Liquid/Semi-solid fresh specimens** – pipette 25 μL of specimen with a transfer pipette and dispense into the Diluent/Conjugate mixture. Use the same transfer pipette to mix the diluted specimen.
3. **Formed/Solid fesh specimens** – Care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 2 mm diameter, the equivalent of 25 μL) of the specimen into the Diluent/Conjugate mixture. Emulsify the specimen using the applicator stick.
4. **Fecal specimens in fixative or transport media** – pipette 100 μL (2 drops from transfer of sample into the *Diluent/Conjugate* mixture.
5. **External Control Samples:**

**External Positive Control –** add four drops of Positive Control (gray-capped bottle) to the appropriate test tube containing Diluent.

**External Negative Control -** add 100 μL Diluent to the appropriate test tube containing Diluent.

**NOTE:** Transferring too little specimen, or failure to mix and completely suspend the specimen in the Diluent/Conjugate mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results due to restricted sample flow.

**TEST PROCEDURE**

1. Obtain one Membrane Device per specimen. The foil bags containing the devices should be brought to room temperature before opening. Use the device immediately after opening. Label each device appropriately and orient it on a flat surface so the “QUIK CHEK” print is located at the bottom of the device, and the small Sample Well is

located in the top right corner of the device.

 

1. Cap each tube of diluted specimen and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube. Once a patient sample or Positive Control has been diluted in the Diluent/Conjugate mixture, it may be stored at 2 - 8°C for any period of time up to 24 hours prior to addition to the Membrane Device.
2. Using a new transfer pipette, transfer 500 μL of the diluted sample-conjugate mixture into the Sample Well (smaller hole in the top right corner of the device) of a Membrane Device, making certain to expel the liquid sample onto the wicking pad inside of the Membrane Device. When loading the sample into the sample well, make sure that the

tip of the transfer pipette is angled towards the Reaction Window (larger hole in the middle of the device).

1. Incubate the device at room temperature for 15 minutes – the sample will wick through the device and a wet area will spread across the Reaction Window.

**NOTE FOR SAMPLES THAT FAIL TO MIGRATE:**

Occasionally, a diluted fecal specimen clogs the membrane and the Reaction Window does not wet properly. This is ordinarily due to the addition of too much fecal specimen to the sample Diluent. If the diluted fecal specimen fails to migrate properly within 5 minutes of adding the sample to the Sample Well (i.e. the membrane in the Reaction

Window does not appear to be completely wet), then add 100 μL (4 drops) of Diluent to the Sample Well and wait an additional 5 minutes (for a total of 20 minutes). If the specimen still fails to migrate, retest the specimen.

1. After the incubation, add 300 μL of Wash Buffer to the Reaction Window using the graduated white dropper assembly (or equivalent). Allow the Wash Buffer to flow through the Reaction Window membrane and be absorbed completely.
2. Add 2 drops of Substrate (white-capped bottle) to the Reaction Window. Read and record results visually after 10 minutes

**INTERPRETATION OF RESULTS**

1. Interpretation of the test is most reliable when the device is read immediately at the end of the reaction period. Read the device at a normal working distance in a well-lit area. View with a line of vision directly over the device. A positive result may be interpreted at any time between the addition of Substrate and the 10-minute read time. However, a test cannot be interpreted as negative or invalid until the 10 minutes following the addition of the Substrate has been completed.
2. Observe the device for the appearance of blue dots in the middle of the Reaction Window representing the internal positive control. Observe device for the appearance of blue lines on either side of the control dots (Giardia on the right, Cryptosporidium on the left). Lines may appear faint to dark in intensity.
3. **Positive Giardia Result** (Fig. 1b, Fig. 1c):

 The control dots and the Giardia line (on the right side of the Reaction Window) are visible. The lines may appear

 faint to dark in intensity - any blue line on the right side of control dots is considered positive. Do not interpret

 membrane discoloration as a positive result. A positive result indicates the presence of Giardia antigen and a

 properly reactive positive control line.

1. **Positive Cryptosporidium Result** (Fig. 1b, Fig. 1d):

 The control dots and the Cryptosporidium line (on the left side of the Reaction Window) are visible. The lines may

 appear faint to dark in intensity - any blue line on the left side of control dots is considered positive. Do not

 interpret membrane discoloration as a positive result. A positive result indicates the presence of Cryptosporidium

 antigen and a properly reactive positive control line.

1. **Negative Result** (Fig. 1a):

Only the control dots in the middle of the Reaction Window are visible. No lines are visible. A negative result indicates that Giardia and Cryptosporidium antigen are either not present in the sample, or are below the detectable limits of this test.

1. **Invalid Result** (Fig. 1e-1h):

The test is invalid if the control dots are not present at the completion of the reaction period (Figures 1e-1h)

**QUALITY CONTROL:**

**Internal:** A dotted blue line must be visible in the middle of the Reaction Window, below the “C” on every Membrane Device that is tested. The appearance of the blue control dots 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 Device. A clear background in the result area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will be white to give a discernible result.

 **Failure of Internal Quality Control**

1. Repeat testing of patient specimen.
2. Repeated failure – Do not report patient results - Contact supervisor and Alere North America
3. Perform external quality control.
4. Refer specimen to Reference Laboratory for testing

**External:** The reactivity of the GIARDIA/CRYPTOSPORIDIUM QUIK CHEK kit should be verified upon receipt using the Positive Control and negative control (Diluent). The Positive Control is supplied with the kit (gray-capped bottle). The Positive Control confirms the reactivity of the other reagents associated with the assay. Diluent is used for the negative

control.

1. Quality Control is performed with each new lot and/or shipment crosschecking it against the old lot using the old lot controls and diluent or patient samples tested against the old lot.
2. Quality Control is performed every 30 days.

**Failure of External Quality Control**

1. Repeat testing of patient specimen.
2. Repeated failure – Do not report patient results - Contact supervisor and Alere North America
3. Refer specimen to Reference Laboratory for testing

**COMPUTER RESULT ENTRY**

Enter results into the LIS.

**Positive** **Cryptosporidium** specimens must be referred to the Minnesota Department of Health for confirmation. See appendix for guideline for submitting specimens for confirmation of rapid screening results (computer ordered as a reflex test - MNCRY).

**LIMITATIONS**

1. Due to the low Positive Predictive Value (PPV) of rapid cryptosporidium assays, positive results must be interpreted in conjunction with patient clinical history and seasonal prevalence of cryptosporidium (highest June-October. Rapid assays should not be used as the sole method for diagnosing cryptosporidiosis. Major risk factors for cryptosporidium infection are contact with cattle, unpasteurized milk consumption, well water consumption, recreational swimming, or contact with a previously ill individual.
2. The GIARDIA/CRYPTOSPORIDIUM QUIK CHEK test is used to detect Giardia and/or Cryptosporidium in fecal

specimens. The test confirms the presence of antigen in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient.

1. Fecal specimens are extremely complex. Optimal results with the GIARDIA/CRYPTOSPORIDIUM QUIK CHEK test are obtained with specimens that are less than 72 hours old. Most undiluted fresh specimens can be stored between 2°C and 8°C for 72 hours before degradation of the antigen is noted. If specimens are not assayed within this time period, they may be frozen for up to 90 days and thawed. However, repeated freezing and thawing may result in loss in the immunoreactivity of antigen.
2. Some specimens may give weak reactions. This may be due to a number of factors such as the presence of low

levels of antigen, the presence of binding substances, or inactivating enzymes in the feces. Under these conditions, a fresh specimen should be tested.

1. The GIARDIA/CRYPTOSPORIDIUM QUIK CHEK test is qualitative. The intensity of the color should not be

 interpreted quantitatively

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**EXPECTED VALUES** Normal healthy individuals should not be infected with Giardia or Cryptosporidium and should test negative in the GIARDIA/CRYPTOSPORIDIUM QUIK CHEK test. A positive test result in the GIARDIA/CRYPTOSPORIDIUM QUIK HEK test indicates that the person is shedding detectable amounts of Giardia and/or Cryptosporidium antigen.

**BACK-UP PROCEDURE:**

Refer specimen to Reference Laboratory for Giardia Antigen and Cryptosoridium testing

**REFERENCES:**

1. Giardia/Cryptosporidium Quik Chek Package Insert; Alere North America, Inc. Orlando, FL 32810,

Tel. 1-877-441-7200. Component Code #91-407-01 Issued:

1. T.J. Robinson, E.A. Cebelinski, C. Taylor and K.E. Smith, Evaluation of the Positive Predictive Value of Rapid Assays Used by Clinical Laboratories in Minnesota for the Diagnosis of Cryptosporidiosis, Clinical Infectious Diseases 2010;50:e53-e55; 2010
2. PARA-PAK package insert, Meridian Bioscience, Inc.,Cincinnati, OH 45244, Rev8/03

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**Appendix**

**Submitting Specimens for Confirmation of Rapid Screening Results for Cryptosporidium to MDH**

**Acceptable specimens**

* Prefer stool preserved in formalin and PVA
* Stool in preservatives intended specifically for parasitology examination.

**Form Required**

1. Fill out MDH EIP report form.
2. Project **#332**
3. Enter patient and facility information.
4. Enter specimen information.
5. **Do Not** check the Reportable Disease Rule Box.
6. Under “Test Requested” check the box for “Parasite/ID Confirmation”. Specify Cryptosporidium.
7. No fee sticker is required.

**Storage and Transport**

Preserved specimens may be stored and transported at room temperature.

**Reporting**

* Presence will be reported as “Oocysts of Cryptosporidium present”
* Absence will be reported as “Oocysts of Cryptosporidium not found”
* Positive samples will automatically be referred and reported according to the reporting rule.

**Reference**

Guidelines for Cryptosporidium Specimen Submission to the Minnesota Department of Health (August 2010),

Minnesota Dept. of Health, St. Paul, MN